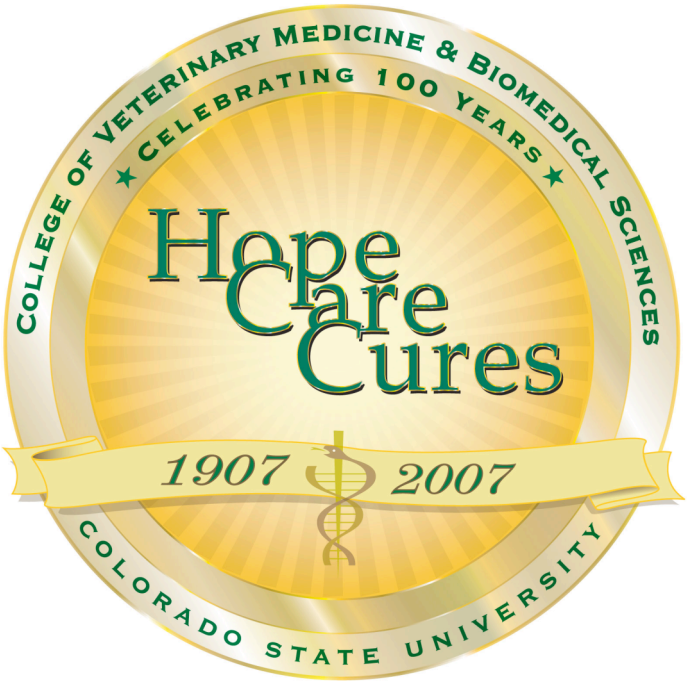
Colorado State University
College of Veterinary Medicine and Biomedical Sciences

8th Annual CVMBS Research Day



Scientific Proceedings

The Hilton Hotel
February 17, 2007

Presented by:

College of Veterinary Medicine and Biomedical Sciences

The Society of Phi Zeta
The Honor Society of Veterinary Medicine

CVMBS Research Day 2007

Schedule Of Events		Room
12:30-1:00	Poster set up	Oklahoma
1:00	Opening remarks – Dr. Terry Nett	Idaho/Michigan
1:05	Keynote speaker – Dr. Jennifer M. McCallum *Legal and Ethical Considerations Regarding the Use of Stem Cell in the U.S. and Abroad*	Idaho/Michigan
1:50	Pfizer Research Award Winner, Dr. Brian Foy *Killing the Messenger: Mosquitocidal Strategies for Disease Control*	Idaho/Michigan
2:15-5:45	Oral Presentation I Graduate Student: Basic Sciences Moderators:	Idaho
2:15-5:45	Oral Presentation II Graduate Student: Clinical Sciences PVM: Basic/Clinical Sciences Moderators:	Michigan
3:00-4:00	Poster Session I Judging: Graduate Students/Post-doc Basic and Clinical Sciences	Oklahoma
4:00-5:00	Poster Session II Judging: PVM/Faculty Basic and Clinical Sciences	Oklahoma
3:00 – 6:00	Posters on Display & Sponsor Exhibits	Oklahoma
5:45 – 6:30	Social Hour, Remove Posters	Oklahoma
6:30	Awards	Oklahoma

Oral Presentation: - Please limit to a 12 minute talk with 1-3 minutes for questions and changeover. Oral presentations will be in the Idaho and Michigan Rooms.

Poster Presentation: - Please hang your posters on Feb. 17 from 12:30-1:00 in the Oklahoma. Individuals presenting the poster must be in attendance to discuss their materials with judges from 3-5 pm.

KEYNOTE SPEECH

CVMBS Research Day
Saturday, February 17th, 2007

Dr. Jennifer M. McCallum

Legal and Ethical Considerations Regarding the Use of Stem Cell in the U.S. and Abroad

Idaho/Michigan Ballrooms
The Hilton Hotel
Fort Collins, CO

Dr. McCallum is an advisor to Grayson & Associates. She is a Patent Attorney with a practice focusing primarily on biotechnology, biomedical and medical device clients in a wide variety of areas including agricultural biotechnology, pharmaceutical chemistry, genetic engineering, medical diagnostics, medical devices, genomics and proteomics.

She provides Grayson & Associates and her clients many aspects of counseling such as U.S. and foreign patent prosecution matters, patentability searches and opinions, and infringement analysis, as well as transactional matters such as technology transfer and licensing agreements.

Dr. McCallum has a Ph.D. in Reproductive Physiology from Colorado State University, a J.D. from the University of Colorado and is a registered Patent Attorney with the United States Patent and Trademark Office.

PFIZER RESEARCH AWARD WINNER

CVMBS Research Day
Saturday, February 17th, 2007

Dr. Brian Foy

Killing the Messenger: Mosquitocidal Strategies for Disease Control

Idaho/Michigan Ballrooms
The Hilton Hotel
Fort Collins, CO

Dr. Foy is an assistant Professor in the Department of Microbiology, Immunology, and Pathology. Dr. Foy studies vector biology and the interactions of vectors with their hosts and with vector pathogens. While much of his research employs molecular, proteomic and genomic techniques, he strives to develop these studies and techniques into practical applications for controlling arthropod-borne diseases. Dr. Foy maintains a laboratory on the main CSU campus for molecular, immunological, and *in vitro* cell culture studies; his laboratory's work involving infecting mosquitoes with pathogens is located at the Arthropod-borne and Infectious Diseases Laboratory and in our BSL-3 laboratories on the CSU Foothills campus.

Oral Presentations

SESSION 1: Idaho Room Moderator: Gopi Palanisamy			
Graduate Students: Basic Sciences			
2:15	Ryan Ashley	An ovine membrane progesterone receptor that mediates calcium mobilization	BMS
2:30	Barbara Biller	Doxorubicin Activates Dendritic Cells and Enhances Antigen Presentation	MIP
2:45	Nicole Garneau	The Influence of the 3' Untranslated Region on Sindbis Viral RNA Stability	MIP
3:00	Patti Kiser	IL-10 prevents anemia during malaria infection	MIP
3:15	Timothy Kurt	Enhanced Detection of Chronic Wasting Disease Prions by Protein Misfolding Cyclic Amplification	MIP
3:30	Jes Kuruvilla	Dengue Virus Infection and Immune Response in Humanized Rag2-/-γc-/- Mice	CMB
3:45	Julie Moreno	Manganese potentiates cytokine-induced NF-kB activation via multiple convergence signaling pathways in astroglia	CMB
4:00	Nicole Nemeth	Dynamics of passive immunity to West Nile virus in domestic chickens	MIP
4:15	BREAK		
4:30	Janet Petty	Glucose Transporter-1 Expression in Canine Osteosarcoma	CS
4:45	Joseph Sottnik	Exploring the Link between Infection and Tumor Inhibition	CS
5:00	Debora Stump	Genotype Rather Than Peripheral Mononuclear Cell Population Dynamics Determines Viral Kinetics in Simian Immunodeficiency Virus Infection	MIP
5:15	Andrea Torres	Insight into feline leukemia virus:host relationships by quantitation of viral and proviral loads.	MIP
5:30	Lance U'Ren	Endogenous Production of Type I Interferons Suppresses Macrophage Accumulation in Tumors	MIP

Oral Presentations

SESSION 2: Michigan Room Moderator: Michael Lund			
Graduate Students: Clinical Sciences			
2:15	Jacquelin Boggs Lawler	Evaluation of di-tri-octahedral (DTO) smectite interaction with Clostridium perfringens alpha, beta and beta-2 exotoxins in vitro.	CS
2:30	Jessica Quimby	The association of Bartonella spp., feline herpesvirus-1, and feline calicivirus infections with feline gingivostomatitis.	CS
2:45	Katie Steneroden	Are veterinarians prepared to deal with contagious respiratory disease in their clinics? Knowledge survey and facility assessment	CS
3:00	Kathryn Vickery	Dose-Escalating Vinblastine Chemotherapy in Canine Mast Cell Tumors	CS
PVM: Basic Sciences			
3:15	Ellie Eschelbach	Estimation of lung lesion burden by MRI and stereology in guinea pigs experimentally infected with M. tuberculosis.	MIP
3:30	Melinda Lopez	Investigating the Efficacy and Toxicity of Two Tirapazamine Analogues in a Nude Mouse Model	SU
3:45	Katie McDermott	Epidermal Growth Factor Promotes the Malignant Phenotype in Canine Hemangiosarcoma	CS
4:00	Brendan Podell	Detection of canine distemper virus in canine blood samples by real-time reverse transcription polymerase chain reaction.	MIP
4:15	BREAK		
PVM: Clinical Sciences			
4:30	Katharine Benedict	Biosecurity Programs at American Veterinary Medical Association accredited Veterinary Teaching Hospitals	CS
4:45	Brandon Fraser	Evidence that elevated Hematocrit and Atrial Natriuretic Peptide are early markers for Cattle at Risk for High Mountain Disease.	CS
5:00	Allison Kean	Use of placental tissue for diagnosis of bovine viral diarrhea virus persistently infected alpaca crias	CS

Poster Presentations

SESSION 1: Post-doc & Graduate Students Basic/Clinical Sciences			
Post-Doc: Basic Sciences			
#1	Ron Carsten	Protection of Mouse Bone Marrow Cells by Resveratrol against Radiation-Induced Chromosome Damage	ERHS
#2	Tracy Davis	Membrane Impermeable Estradiol-Induced Increase in Number of Gonadotropin-Releasing Hormone Receptors in Cultured Ovine Pituitary Cells	BMS
#3	Mark Hughes	La Crosse Virus Vaccine and Immunotherapy: VLPs and CLDCs	MIP
#4	Hend Ibrahim	The role of Nucleophosmin/B23 as a regulator of mRNA metabolism	MIP
#5	Andrew Hartwick	Light-Evoked Responses of Cultured Melanopsin-Expressing Retinal Ganglion Cells	BMS
#6	Stewart Ryan	Simultaneous versus alternate tensioning of wires in a single ring fixator construct	CS
#7	Thomas Welte	Characterization of Antigen Presenting Cells for West Nile Virus Specific T cell Subtypes	MIP
#8	Libin Zhang	Altered expression of CUG-BP in myotonic dystrophy leads to aberrant mRNA decay	MIP
Graduate Student: Basic Sciences			
#9	Andrew Goodyear	Activation of Pulmonary Innate Immunity by Liposome-DNA Complexes provides protection against Burkholderia mallei	MIP
#10	Emily Kampf	Protective efficacy of sub-unit vaccines against aerosol challenge with Francisella tularensis Schu4.	MIP
#11	Lisa Kellihan	In Vitro Model of Pneumonic Burkholderia Infection and Response to Combined Antimicrobial and Immunotherapy	CMB
#12	Phuong Le	Instability in Radiation-Induced Acute Myeloid Leukemia	CMB
#13	Candace Mathiason	Infectious Prions in the Saliva and Blood of Deer with Chronic Wasting Disease	MIP
#14	Scott McCorvey	Combining Pattern Recognition Receptor Agonists for Enhanced Activation of Innate Immunity	MIP
#15	Krystle Reagan	The role of D7 protein in West Nile Encephalitis	MIP
#16	Davis Seelig	The Immunohistochemical Expression of PrPc in, and Experimental Transmission of Chronic Wasting Disease to, a Transgenic Mouse Model.	MIP
#17	Megan Shoemaker	Bovine Viral Diarrhea Virus Infection During Fetal Development	BMS
#18	Kevin Sokoloski	Multiple sequence elements within the VEE 3'UTR repress poly(A) shortening.	MIP
#19	Jesse Thompson	Using Chimeric FIV Constructs to Assess Virulence Determinants.	MIP
#20	Aida Ulloa	Specific role for hTrpC4 in signal-regulated calcium entry in the human myometrium.	BMS
#21	Greg Wilkerson	Detection, Containment and Elimination Strategies for Mouse Parvovirus: A Contemporary Managerial Approach	LAR
#22	Luke Wittenburg	Sodium Valproate to Enhance Doxorubicin Sensitivity	CS

Poster Presentations

Graduate Student: Clinical Sciences			
#23	Danielle Bayliss	Prevalence of Rickettsia species infections in cats with and without fever	CS
#24	Kelvin Kow	Impact of telomerase status on canine osteosarcoma patients	CS
#25	Christianne Magee	Evaluation of Kisspeptin in the Hypothalamic Pituitary Gonadal Axis in the Mare	CS
#26	Gopinath Palanisamy	Disease severity as a measure of M. tuberculosis virulence in the guinea pig model of tuberculosis	MIP
#27	Miranda Spindel	Pradofloxacin for the treatment of feline rhinitis	CS
#28	Jacqueline Whittemore	Association of microalbuminuria and the urine albumin:creatinine ratio with systemic disease in cats	CS

Poster Presentations

SESSION 2: PVM Basic/Clinical Sciences			
PVM: Basic Sciences			
#29	Donald Chung	Therapeutic Targeting of Ire1 in Solid and Blood Tumors	CS
#30	Charity Corning	Equine Kisspeptin and the Equine Hypothalamic-Pituitary Axis	BMS
#31	Kyshia Davis	The Use of Cancer Related Anemia as a Prognostic Indicator of Survival Outcome in a Retrospective Study of 100 Dogs with Osteosarcoma	BMS
#32	Sally Embrey	A stereological study of the uterine stroma in women with premature ovarian failure undergoing hormone replacement therapy.	CS
#33	Brittney Fierro	Development of an in vitro model for assessing enterocytes and lamina propria macrophages during development of spontaneous and bacteria-induced colitis.	MIP
#34	Lindsey Habermann	The Effect of Feline Immunodeficiency Virus on CD40 Ligand Response in Bone Marrow Derived Dendritic Cells	MIP
#35	Eric Hutchinson	The Effects of Rearing Condition and Enrichment on Laboratory Mouse Immune Response, Health, and Behavior	MIP
#36	Claire Reisenhauer	Transplacental infection of bovine fetuses with non-cytopathic bovine viral diarrhea virus type II (BVDV-II): viral spread and brain lesions.	MIP
#37	Lauren Taraba	Effect of iron overload on the pathogenicity of M. tuberculosis in the guinea pig.	MIP
#38	Michelle Truk	Recombinant Bovine Trypsin made in Maize Inactivates Bovine Herpes Virus-1 Adsorbed to the Bovine Zona Pellucida	BMS
#39	Shayna Warner	Lectin dependent phagocytosis of virulent, type A F. tularensis.	MIP
PVM: Clinical Sciences			
#40	Netia Abercrombie	Analysis of Dysplastic Cell Characteristics Found in Bone Marrow of Hematologically Normal Dogs	BMS
#41	Schyler Hiibel	Early Postpartum Biochemical Parameters Related to Dairy Cow Removal	CS
#42	Leilani Ireland	Biopsy-based 1-H and 31-P NMR shows regional metabolic heterogeneity within canine lymphoma	CS
#43	Katheryne Kasper	Development of a real-time PCR assay for the detection of pathogenic leptospires in canine urine	CS
#44	Anneke Lothridge	Effects of GnRH Immunization on Reproduction and Behavior in Female Rocky Mountain Elk	BMS
#45	Kelly McCord	Neutrophil function in septic dogs	CS
#46	Laura Parsley	The lack of a stress leukogram as an indication of Addison's disease in the dog, a retrospective case study	BMS
#47	Audrey Ruple	Nosocomial Syndrome Surveillance	CS
#48	Stacey Sonnenshein	The association between causes of persistent hypercalcemia with the subsequent development of azotemia in dogs	MIP

Poster Presentations

Faculty			
#49	Gerrit Bouma	Identification of candidate genes involved in human XY abnormal gonad development.	BMS
#50	William Dernell	Technetium-99M-Sestamibi Scans to Predict Outcome in Canine Osteosarcoma	CS
#51	Abby Jones	Efficacy of a Non-Replicating Subunit Vaccine Against Pulmonary Challenge with Virulent Yersinia pestis	MIP
#52	Amy Rodriguez	Plasma biochemistry values in dogs anesthetized for cardiopulmonary bypass – influence on anesthetic mortality	CS

Departmental Abbreviations

BMS: Biomedical Sciences
CMB: Cell and Molecular Biology Program
CS: Clinical Sciences
ERHS: Environmental and Radiological Health Sciences
LAR: Laboratory Animal Resources
MIP: Microbiology, Immunology, and Pathology
SU: Stanford University

Thank you to our moderators and judges!!

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Session I ~ Idaho Room

Graduate Students: Basic Science

An ovine membrane progesterone receptor that mediates calcium mobilization.

RL Ashley, CM Clay, T Farmerie, GD Niswender, and TM Nett

Classically, progesterone (P4) has been thought to act only through the well-known genomic pathway, involving hormone binding to nuclear receptors and subsequent modulation of gene expression. However, there is increasing evidence for rapid, nongenomic effects of P4 and the likelihood of a membrane PR (mPR) causing these events is quite plausible. We recently isolated and characterized an ovine mPR distinct from the nuclear PR. The ovine mPR is a 350 amino acid protein that, based on predicted structural analysis, possesses seven transmembrane domains typical of G-protein-coupled receptors and is expressed in the hypothalamus, pituitary, uterus, ovary and corpus luteum. In CHO cells that overexpress a mPR-GFP fusion protein the ovine mPR was uniquely localized to the endoplasmic reticulum and not the plasma membrane. Given the unique localization of the mPR in the endoplasmic reticulum, we hypothesized that ligand stimulation of this receptor would increase intracellular Ca2+ mobilization. As such, the objective of this study was to determine if the ovine mPR alters intracellular Ca2+ concentrations after addition of progestins. There was a rapid increase in free intracellular Ca2+ concentrations ($P < 0.05$) after addition of P4 or 17α- hydroxyprogesterone to CHO cells expressing ovine mPR. Since these experiments were conducted in Ca2+-free medium, the rise in intracellular Ca2+ was believed to originate from the endoplasmic reticulum. To substantiate this hypothesis, cells were treated with thapsigargin to deplete Ca2+ stores from the endoplasmic reticulum. Addition of either progestin to CHO cells expressing ovine mPR after thapsigargin treatment did not result in an increase in intracellular Ca2+, suggestive of progestin action at the endoplasmic reticulum. The increase in Ca2+ appears to be specific to progestins since treatment with estradiol, testosterone, cortisol or RU486 did not evoke an increase in intracellular Ca2+ in CHO cells transfected with mPR.

Doxorubicin Activates Dendritic Cells and Enhances Antigen Presentation

BJ Biller, SW Dow

Purpose: Immunotherapy-based approaches to the treatment of cancer, such as the administration of vaccines targeted to specific tumor antigens, will likely be most effective when given in combination with conventional therapies such as cytotoxic chemotherapy. Little is known, however, about the effects of chemotherapy on many aspects of immunity. For example, it is not known how chemotherapy affects the function of dendritic cells (DCs), which are central mediators of the innate immune system and critical to the development of effective antitumor immunity. Therefore, we investigated the effects of the commonly used chemotherapy drug doxorubicin (DOX) on dendritic cell function in normal mice and in mice with B16 melanoma. Material and Methods: The effect of DOX on costimulatory molecule expression was assessed by immunostaining and flow cytometry of bone-marrow derived DCs. The in vivo effects of DOX were assessed on splenic DC from i.v. injected mice. In addition, the effects of DOX on antigen presentation by DCs were evaluated by assessment of allogeneic T cell proliferation in vitro or in vivo. The effects of treatment with DOX on the number and phenotype of DCs within the spleen, lymph nodes and tumor tissues of mice with established melanomas were also determined. Results: Treatment with DOX significantly upregulated costimulatory molecule expression and antigen presentation by DCs, following both in vitro and in vivo DOX administration. In mice with tumors, treatment with DOX increased the number of activated DCs within tumor-draining lymph nodes and within tumor tissues themselves. Conclusions: Our results indicate that DOX is uniquely effective amongst other common chemotherapy drugs in augmenting the ability of DCs to present antigens. These findings suggest an important rationale for the inclusion of DOX in combined chemotherapy and immunotherapy protocols for prevention or treatment of cancer.

The Influence of the 3' Untranslated Region on Sindbis Viral RNA Stability

NL Garneau, KJ Sokoloski, CJ Wilusz & J Wilusz.

Purpose: To determine the half-life of Sindbis viral RNA in both mammalian and mosquito host cell types, and to elucidate the sequence elements in the viral 3'UTR responsible for stability. Materials/Methods: We have developed an innovative system to visualize viral RNA decay in living cells to permit us to measure the contribution of the Sindbis 3'UTR as a stabilizing element during an infection. This assay utilizes a temperature sensitive mutation in the viral RNA polymerase to allow inhibition of viral transcription. We can then follow decay of the subgenomic viral RNA by RNase protection assay at various times after transcription inhibition. To examine potential stability elements in the 3'UTR we use an in vitro deadenylation assay derived from extracts of C6/36 Aedes albopictus cells. Results: The subgenomic RNA of Sindbis virus is moderately stable in both mammalian and mosquito host cell types, with a half life of six and three hours, respectively. Using an in vitro assay we have been able to show that the 3'UTR of Sindbis virus is capable of inhibiting the first step of mRNA decay; deadenylation. This effect is mediated principally by the Repeat Sequence Elements. Conclusions: Sindbis viral subgenomic RNA is moderately stable suggesting that the virus has evolved a means of evading the host RNA decay machinery. This evasion appears to be due to the Repeat Sequence Elements located in the viral 3'UTR, which prevent deadenylation, the rate-limiting step of RNA decay.

IL-10 prevents anemia during malaria infection

P Kiser, C Olver, A Avery

Purpose: Malaria is caused by a protozoan parasite that infects red blood cells (RBC). A major cause of morbidity and mortality in endemic areas is severe anemia, which is not proportional to the degree of parasitemia. The purpose of this study was to determine if anemia during malaria infection is mediated by pro-inflammatory cytokines, and to investigate the mechanism of anemia. Materials and Methods: IL-10 knockout (IL-10 KO) mice produce high levels of pro-inflammatory cytokines during malaria. IL-10 KO and wild type (WT) mice were infected with Plasmodium yoelii and anemia and parasitemia were monitored. Red cell production (erythropoiesis) and red cell destruction in IL-10 KO and WT mice were monitored by enumerating RBC precursors and RBC clearance, respectively, in both strains of mice. Results: IL-10 KO mice effectively controlled their parasitemia while experiencing more severe anemia than the WT mice. The mechanism of anemia appears to be increased erythrocyte clearance, because erythropoietic responses by both strains were similar. Conclusions: Anemia during malaria infection is caused by direct RBC destruction as well as increased destruction of uninfected RBC. IL-10 KO mice, which produce high levels of pro-inflammatory cytokines during malaria infection, have a greater degree of anemia than WT mice. Our findings complement studies of human populations infected with malaria, which have shown that a high ratio of the pro-inflammatory cytokines TNFa and IL-12 to IL-10 is associated with more severe anemia. Our results have implications for the mechanism of anemia in other diseases involving erythrocyte parasites.

Enhanced Detection of Chronic Wasting Disease Prions by Protein Misfolding Cyclic Amplification

TD Kurt, MR Perrott, CJ Wilusz, J Wilusz, S Supattapone, and EA Hoover

Purpose: Chronic Wasting Disease (CWD) is a fatal Transmissible Spongiform Encephalopathy (TSE) of cervids. TSEs are associated with the conversion of the normal prion protein (PrPC) to the disease-associated misfolded isomer (PrPRES). Our goal was to apply protein misfolding cyclic amplification (PMCA) to amplify CWD PrPRES in vitro. This capability would have many potential impacts including (1) detection of very low levels of PrPRES in various tissues and biological fluids and (2) estimation of the potential for cross-species infection by CWD. Materials/methods: PMCA was performed by spiking normal (CWD-negative) brain homogenates with serial dilutions of brain homogenate from CWD-positive deer. The mixtures were incubated at 37 degrees for 48 to 72 hours in an automated sonicator which delivered 40 second pulses every 30 minutes. After sonication/incubation, the mixtures were digested with proteinase K to degrade normal cellular proteins including PrPC. Protease-resistant PrPRES was then detected by immunoblotting. Results: PMCA produced up to 200-fold increases in PrPRES, relative to input quantity, after one round of sonication/incubation, and up to 2 million-fold increases after three sonication/incubation cycles--representing a 400,000-fold improvement over previous, non-PMCA amplification results. Moreover, PMCA demonstrated amplification of CWD PrPRES using homogenates from non-neural tissues, such as testis, not previously shown to amplify CWD in vitro, suggesting that PMCA could provide insight into the potential for sexual transmission of CWD. Lastly, CWD PrPRES was amplified using brain homogenates from non-cervid species, suggesting that PMCA may be useful in investigating the CWD species barrier. Conclusions: (1) PMCA made possible up to 2 million-fold more sensitive detection of the CWD-associated abnormal prion protein in vitro. (2) PMCA offers promise in providing insight into both mechanisms of transmission and the species barriers in CWD infection.

Dengue Virus Infection and Immune Response in Humanized Rag2-/-γc-/- Mice

JG Kuruvilla, RM Troyer, S Devi,RK Akkina

Dengue viral pathogenesis and vaccine studies have been hampered by the lack of an animal model that can faithfully recapitulate the human disease and immune response. To overcome this major hurdle we employed a novel mouse model that permits multi-lineage human hematopoiesis and immune response following transplantation with human blood forming stem cells. To generate immunocompetent humanized mice, neonatal Rag2-/- gamma chain-/- mice were engrafted with human CD34+ hematopoietic stem cells by intrahepatic injection (referred to hereafter as RAG-hu mice). Transplanted mice showed human cell engraftment levels of up to 85% in white cell fractions of peripheral blood as well as significant engraftment in the thymus, spleen, liver, lymph nodes and bone marrow as assessed by FACS for the human CD45 pan-leukocyte marker. There was de novo development of major functional cells of the human adaptive immune system including human macrophages, B cells, T cells and dendritic cells. These humanized mice were challenged with a pool of Dengue-2 viral strains including primary isolates to evaluate their capacity to sustain a productive viral infection. Virus replication was monitored by real-time RT-PCR on plasma samples collected every two days post infection. Infected humanized mice showed fever and plasma viral loads reaching up to 1 million copies/ml that were sustained for up to three weeks. Presence of human anti-Dengue antibodies were evaluated using an antibody capture ELISA. Anti-Dengue IgM could be first detected by two weeks post-infection followed by IgG at six weeks demonstrating human antibody class switching. Furthermore, sera from some of the infected mice were found to be capable of Dengue virus neutralization. These results have shown for the first time that humanized mice are capable of Dengue viral primary human immune responses, thus paving the way for new Dengue immunopathogenesis and vaccine studies.

Manganese potentiates cytokine-induced NF-kB activation via multiple convergence signaling pathways in astroglia

JA Moreno, RB Tjalkens

Manganism is a neurodegenerative disease affecting the basal ganglia with concomitant astrocytosis, which can produce neurotoxic levels of inflammatory mediators such as nitric oxide (NO) and pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF) and interferon-gamma (IFN). It has been hypothesized in the present studies that upstream signaling events involving MAP kinases and soluble guanylyl cyclase (sGC) pathways underlie the capacity of manganese (Mn) to enhance cytokine-dependent activation of NF-kappa B (NF-kB). Coexposure of primary astrocytes to 10 micromolar Mn and TNF/IFN significantly potentiated both steady-state levels of nitric oxide synthase 2 (NOS2) mRNA and production of NO ($p<0.001$). In order to determine NF-kB's role a dominant negative mutant of IkB-alpha was used and significantly decreased NOS2 mRNA and NO production which was normally induced by co-exposure of Mn and cytokines ($p < 0.001$). The intensity of GFP fluorescence driven by the NF-kB promoter was measured in transgenic astrocytes and was markedly increased with both Mn and cytokine exposure, indicating the upstream role of NF-kB. Phosphorylation of ERK and IkBa rapidly increased upon exposure to Mn and TNF/IFN, but was slightly abrogated by pretreatment with a blocking antibody to beta 1 integrin and significantly inhibited by both an ERK and sGC inhibitor, respectively. These data indicate that low concentrations of Mn potentiate cytokine-induced NO production and expression of NOS2 protein and mRNA through activation of sGC, beta1 integrin and subsequent ERK-dependent enhancement of NF-kB signaling.

Dynamics of passive immunity to West Nile virus in domestic chickens

NM Nemeth, RA Bowen.

Purpose: Birds are the principle amplifying hosts for West Nile virus (WNV) and understanding the acquisition and decay of passive immunity is important to avian surveillance and diagnostics. We characterized passive transfer of WNV-neutralizing antibody from domestic chicken hens to eggs and chicks, and protective efficacy and decay of maternally-acquired antibody. We also characterized age-associated changes in the magnitude of viremia and examined the possibility of vertical WNV transmission. Materials/methods: Antibody titers were determined by plaque reduction neutralization test and viral titers by Vero cell plaque assay. Results: All egg yolks and chicks from seropositive hens were maternal antibody positive. Maternal antibodies were undetectable in most chicks by 28 days post-hatch (DPH), but some chicks remained protected from viremia as late as 42 DPH. Most chicks challenged at 42 DPH or later seroconverted by 10 days post-inoculation (DPI). By 56 DPH, chicks from immune hens had viremia profiles similar to control chicks. There were significant age-related differences in WNV-attributed morbidity and viremia of unprotected chicks. In unprotected chicks, antibodies in response to infection were first detected between 7-10 DPI. Vertical transmission of WNV was not detected. Conclusions: These results aid in the interpretation of wild bird serosurveys, as well as epidemiological data involving the distribution of WNV antibodies in birds of varying age groups. Chicks with maternal antibody are protected for a limited time after hatching, which likely coincides with the time that they are most susceptible to high-level viremia and morbidity from WNV infection. Seropositive chicks of <7 DPH likely have maternal antibody and do not signify WNV transmission. The dynamics of maternal antibody decay and subsequent immunological naïveté of previously maternal antibody positive birds are additional factors that affect WNV transmission and population health of birds.

Glucose Transporter-1 Expression in Canine Osteosarcoma

JC Petty, EJ Ehrhart, SE Lana, DH Thamm, JB Charles, AM Bachand

Purpose: Hypoxia in tumors has been associated with an increased resistance to radiation and chemotherapy, and an increase in metastatic rate. This may be due to expression of proteins in the Hypoxia Inducible Factor-1 alpha (HIF-1 alpha) pathway. HIF-1 alpha is a transcription factor induced by hypoxia. Glucose Transporter-1 (GLUT-1), a glucose transporter, is a downstream product of HIF-1 alpha pathway activation and has been shown to be over expressed in a variety of human tumors. The purpose of this study was to determine if GLUT-1 is expressed in canine osteosarcomas and if expression is related to tumor necrosis or outcome. Materials/methods: Immunohistochemistry was performed on 44 histologically confirmed osteosarcoma tissue samples to assess expression of GLUT-1. Normal canine tissues shown to express GLUT-1 in humans were used as positive controls. The samples were evaluated by one author. GLUT-1 was evaluated by a set scoring method for percent of cells staining positive, the intensity of staining, and a product of the two scores for each sample. The tissues were evaluated on H&E slides for percent necrosis. Results: Of 44 cases, 27 (61%) expressed GLUT-1. For percent necrosis, 39 of 44 cases were available for evaluation. There was no statistical correlation between GLUT-1 and disease free interval, survival time, or percent necrosis. Conclusion: As hypothesized, GLUT-1 is present in canine appendicular osteosarcomas. Though there was no statistical correlation with disease free interval, survival time and necrosis, this could be due to small sample size. A more objective evaluation of GLUT-1 and other proteins in the HIF-1 alpha pathway may be warranted. A future direction includes evaluation of GLUT-1 expression as a therapeutic target of hypoxic tumor cells.

Exploring the Link between Infection and Tumor Inhibition

JL Sottnik, LW U'Ren, DH Thamm, SD Dow.

Purpose: Osteosarcoma (OS) is the most prevalent malignancy associated with bone in humans and dogs, and the high metastatic rate of these tumors leads to high morbidity rates. The limb-spare procedure has helped dogs and humans with OS retain their limbs, however, the control of metastases remains the primary cause of death. It was discovered that patients developing an infection at their surgical site lived twice as long, and their metastases took twice as long to develop. The goal of this project is to develop a mouse model of bacterial infection that can be used to probe the mechanisms underlying these observations. Materials/Methods: Balb/c and C57Bl/6 mice were infected with a strain of S. aureus transfected to express luciferase. The bacteria were lyophilized and implanted into the intermedullary cavity of the tibia. Syngeneic OS and melanoma cell lines were then injected subcutaneously into the mice after infection. Mice were imaged to track the extent of infection, and tumor measurements were taken to evaluate tumor growth. Survival was considered from the time of tumor injection until 1 cm longest tumor diameter, at which time the mouse was sacrificed and terminally bled. ELISA's for interferon-gamma and vascular endothelial growth factor (VEGF) were performed to assess changes in cytokine production. Results: Mice with infected suture implanted into their tibia have had a significantly increased survival over mice undergoing a sham operation with sterile suture implanted. ELISA results are pending. Conclusions: Mice with localized bone infections had a significant tumor growth delay, and survived significantly longer than non-infected mice. These data closely resemble the clinical observations made in dogs and humans with OS and infected limb allografts. Therefore, we conclude that the mouse bone infection model reproduces many of the clinical observations and will therefore be very useful for mechanistic studies of antitumor activity.

Genotype Rather Than Peripheral Mononuclear Cell Population Dynamics Determines Viral Kinetics in Simian Immunodeficiency Virus Infection

DS Stump, R Gautam, C Apetrei, S VandeWoude

Purpose: Rhesus macaques of Chinese and Indian origin were challenged with a pathogenic Simian Immunodeficiency Virus as part of a study to assess alloimmunization as an HIV vaccine strategy. All animals became infected and viral loads and peripheral blood mononuclear cell (PBMC) population dynamics were determined in order to find correlates for the differences in viral kinetics. Materials/methods: Blood was collected at six time points post challenge and processed within 3 hours. RNA from anticoagulated plasma was directly extracted and quantitated by PCR immediately or stored at –80oC. Immunophenotyping was performed by multicolor flow cytometry on peripheral blood. One hundred µl of whole blood was stained with 10 monoclonal antibodies to detect the main cell populations. General immunophenotyping of all lymphocytes, monocytes and granulocyte cell subsets together with naïve, central and effector memory marker expression on T cell subsets and chemokine receptor expression on T cell subsets was determined using fluorescently labeled antibodies to CD3, CD8, CD4 CD20, CCR5, Fas, CD28, CXCR4, CD14, and CCR2 known to cross react with rhesus proteins. Results: Viral loads for all animals peaked by day 14 post inoculation (pi) with 7 of 8 animals having viral titers between 10^6 and 10^7 viral copies per ml. The Chinese rhesus showed significant reduction in viral loads by day 45 pi compared to the Indian rhesus. All of the Indian rhesus were euthanized due to clinically apparent disease by day 91 pi whereas none of the Chinese rhesus exhibited clinical disease. There were no consistent PBMC population changes correlating with viral load or clinical outcome. Conclusion: Chinese rhesus macaques are able to control viral replication and resist clinical illness when challenged with pathogenic SIVmac239 whereas Indian rhesus macaques can not. Resistance does not correlate with PBMC or target cell phenotype dynamics.

Insight into feline leukemia virus:host relationships by quantitation of viral and proviral loads.

AN Torres, KP O'Halloran, LJ Larson, RD Schultz, EA Hoover.

Purpose: Here we ask whether feline leukemia virus (FeLV) DNA is transcriptionally active in the absence of antigenemia and whether detectable viral RNA represents infectious virus.
Materials/methods: We developed and validated a quantitative real-time polymerase chain reaction assay (qPCR) to detect FeLV RNA. We then applied this methodology, together with viral DNA qPCR, FeLV p27 capsid antigen capture enzyme-linked immunosorbent assay (ELISA), and a viral infectivity assay, to examine groups of vaccinated (4 groups, n=8 per group) and unvaccinated (n=8) cats challenged with FeLV. Results: The viral RNA qPCR assay proved to be highly sensitive, specific, reproducible, and allowed reliable quantitation. Two commercially available whole inactivated virus (WIV) FeLV vaccines provided substantial protection against FeLV challenge. In nearly every recipient of these vaccines, neither viral DNA, RNA, antigen, nor infectious virus could be detected in blood. In the remaining cats, circulating viral RNA and DNA levels were highly correlated and in addition, high viral and proviral burdens were associated with detection of infectious virus. The real-time qPCR assays were more sensitive than the most commonly used FeLV diagnostic assay, the capsid antigen capture ELISA. Conclusions: (1) Two FeLV vaccines produced virtual ‘sterilizing immunity’ as documented by our inability to detect the viral nucleic acids in blood post challenge, lending support to the principle that protective immunity to retroviral infection can be produced by WIV immunoprophylaxis. (2) The detection of FeLV RNA reinforced the four host:virus relationships previously defined by viral DNA and antigen detection. (3) FeLV DNA initiates a transcriptionally active infection, even in the absence of detectable antigenemia. (4) Real-time DNA and RNA qPCR assays permit greater depth in understanding of FeLV:host relationships.

Endogenous Production of Type I Interferons Suppresses Macrophage Accumulation in Tumors

L U'Ren, D Kamstock, J Bushanam, S Dow

Purpose: Most tumors contain large numbers of macrophages, which in many cases serve to promote tumor growth by a variety of mechanisms. Several cytokines have been identified that promote the recruitment and accumulation of macrophages in tumors, including CSF-1 and MCP-1. However, factors that actively suppress the accumulation of macrophages within tumors have not previously been identified. Therefore, we investigated the role of type I IFNs (interferon-alpha and interferon-beta) in regulating macrophage survival and accumulation within tumors. Methods: We assessed the effects endogenous production of type I interferons on macrophage accumulation in tumors utilizing mice lacking a functional type I IFN receptor (IFN-a/bR -/- mice). A syngeneic fibrosarcoma cell line (MCA2.1) was grown subcutaneously in wild type or IFN-α/βR -/- mice, and macrophage infiltration was assessed by flow cytometry and immunohistochemistry. In vitro assays utilizing wild type or IFN-a/bR -/- bone marrow macrophages were used to assess the effects of type I interferons on macrophage survivability and proliferation. Results: We found that there were significant increases in the numbers of F4/80+ and CD68+ macrophages in tumors of IFN-a/bR -/- mice, compared to tumors in wild type control mice. Tumors in IFN-a/bR -/- mice also had significantly increased microvessel density. The increase in intratumoral macrophages in IFN-a/bR -/- mice was not due to tumor overproduction of CSF-1 or MCP-1. Rather, in vitro assays indicated that suppression of macrophage responsiveness to CSF-1 by type I IFNs was primarily responsible for the accumulation of macrophages in tumors of mice unable to respond to type I IFNs. Conclusions: These results indicate that endogenous production of type I IFNs by tumor cells or inflammatory cells within tumors may be an important mechanism of suppressing accumulation of tumor-associated macrophages, which may in turn result in suppression of tumor angiogenesis.

Session II ~ Michigan Room

Graduate Students: Clinical Science

PVM: Basic Science

PVM: Clinical Science

Evaluation of di-tri-octahedral (DTO) smectite interaction with Clostridium perfringens alpha, beta and beta-2 exotoxins in vitro.

J Boggs Lawler, DM Hassel, JL Traub-Dargatz, R Magnuson, C Hirota, A Hill, PM McCue

Purpose: Clostridial-associated enterocolitis is a sporadic disease of neonatal foals and adult horses. However, it is associated with a high case-fatality rate and substantial economic losses. The severity of disease appears to be related to the particular Clostridial isolate, and thus, the specific exotoxin produced. Higher levels of Clostridium perfringens alpha, beta, and beta-2 exotoxins have been identified in the feces of clinically affected animals as compared to healthy controls. Di-tri-octahedral (DTO) smectite has been shown to be effective at adsorbing toxins; however, the effect on C. perfringens exotoxins has not been evaluated. The purpose of this study was to determine if DTO smectite effectively decreases detectable Clostridium perfringens exotoxins as compared to bismuth subsalicylate in vitro. Materials/Methods: Alpha, beta, and beta-2 C. perfringens exotoxins were mixed individually with serial dilutions of either DTO smectite or bismuth subsalicylate and then tested for the presence of clostridial toxin by ELISA. Results: DTO smectite decreased the amount of alpha, beta and beta-2 C. perfringens exotoxins detectable in a dose-dependent manner and was significantly more effective than bismuth subsalicylate at reducing toxin in vitro. Conclusions: DTO smectite effectively decreases the amount of detectable C. perfringens exotoxins in vitro. Di-tri-octahedral smectite appears to be a reasonable adjunctive therapy for equine clostridiosis; however in vivo studies are necessary to assess clinical application.

The association of Bartonella spp., feline herpesvirus-1, and feline calicivirus infections with feline gingivostomatitis.

JM Quimby, T Elston, C Critchfield, JR Hawley, M Brewer, AK, Miller, MR Lappin.

Purpose: Gingivostomatitis (GS) is a significant clinical condition in cats due to severe oral discomfort and progression of periodontal disease. Several infectious agents have been associated with GS, but the causal relationship remains unclear. The purpose of this study was to perform a standardized infectious disease diagnostic workup in a group of cats with and without GS in an attempt to determine infectious disease associations. Materials/Methods: The cats used in this study were group-housed and had flea exposure. At the time of blood, serum, and oral swab collection, cats were classified as normal or having active GS. Serum was tested for FeLV antigen and antibodies against FIV, feline calicivirus (FCV), feline herpesvirus 1 (FHV-1), and Bartonella species (ELISA and western blot immunoassay [WB]). PCR assays that amplify the DNA of Bartonella species and FHV-1 and a reverse transcriptase PCR (RT-PCR) assay that amplifies the RNA of FCV were performed on DNA or RNA extracts from blood and throat swabs. Prevalence rates of each organism and predictive values for each assay were calculated. Results: Nine cats had active GS, 36 were normal (N), and all were negative for FeLV and FIV. Prevalence rates for Bartonella species antibodies by ELISA (GS = 44.4%; N = 44.4%)(did they both have the same prevalence, or is this a typo?), Bartonella antibodies by WB (GS = 22.2%; N = 66.7%), FHV-1 antibodies (GS = 100%; N = 94.4%), FCV antibodies (GS = 100%; N = 100%), Bartonella PCR assay on blood (GS = 11.1%; N = 0%), Bartonella PCR assay on oral swabs (GS = 11.1%; N = 11.1%), FHV-1 PCR assay on oral swabs (GS = 0%; N = 8.3%), and FCV RT-PCR assay on oral swabs (GS = 0%; N = 8.3%) varied amongst the groups. Predictive values varied greatly between assays Conclusions: Results of these assays failed to correlate with the presence or absence of GS in the group of cats studied. Additional work is needed to determine the cause of GS in cats and to design optimal diagnostic testing for individual cats.

Are veterinarians prepared to deal with contagious respiratory disease in their clinics? Knowledge survey and facility assessment

K Steneroden, A Hill

Purpose: Small and mixed animal veterinary practices in Colorado will be surveyed to assess their ability to contain contagious respiratory diseases. Veterinarians will be surveyed regarding knowledge of control and spread of contagious respiratory disease. Veterinary technicians will be surveyed with respect to protocols and practices for patients exhibiting signs consistent with contagious respiratory disease. Veterinary practice facilities will be assessed in a subset of veterinary clinics for presence or absence of isolation facility, use of barrier protection, hand washing areas and traffic flow. This project will also provide the opportunity for sophomore veterinary students to be involved in an epidemiological research project where they can learn about and give input on survey/assessment design, visit local veterinary practices, conduct facility assessments and analyze epidemiological data. This project took place over the fall semester 2006. Methods: All veterinary clinics that are members of the Colorado Veterinary Medical Association will be contacted by letter and asked to participate in the study. Second year veterinary students will make telephone contact with the veterinary practices, set appointments and conduct assessments over the fall semester 2006. Results: Preliminary data will be available by the date of the research symposium. We hypothesize that veterinary clinic isolation facilities and their ability to contain contagious respiratory disease will vary by practice size, location, practice type and age of facilities. Conclusion: Preliminary data will be available by the date of the conference.

Dose-Escalating Vinblastine Chemotherapy in Canine Mast Cell Tumors

KR Vickery, DH Thamm, H Wilson, DM Vail

Purpose: Prednisone/vinblastine chemotherapy is efficacious for some canine mast cell tumors (MCT). Reported protocols utilize a dosage of 2.0 mg/m2 vinblastine. Preliminary studies suggest that a higher dosage may be well tolerated, possibly enhancing drug efficacy. The purpose of this study was to evaluate short-term adverse events (AEs) in dogs with MCT receiving prednisone and dose-escalating vinblastine. Materials/methods: Twenty-four dogs were treated with intravenous vinblastine starting at 2.0 mg/m2 then escalating in weekly increments to 2.33 mg/m2, 2.67 mg/m2, and 3.0 mg/m2. Nine patients had concurrent local radiation therapy. AEs were graded using the VCOG-CTCAE v1.0. Results: No dogs receiving 2.0 or 2.33 mg/m2 experienced a Grade 3/4 AE. 9.5% and 5.9% Grade 3/4 AEs occurred at dosages of 2.67 and 3.0 mg/m2. Serious AEs included neutropenia (n=3) and vomiting (n=1), only 1 of which required hospitalization. Three dogs had dosage reductions, one had a dosage reduction and delay, and the owner of another declined further chemotherapy. Although response was a secondary endpoint, the responses of twelve dogs receiving only prednisone/vinblastine chemotherapy were evaluated. A 33.3% response rate (3 CR, 1 PR) was achieved. The median progression free interval in these dogs was 51 days (range 30-136). Conclusions: These data indicate that vinblastine chemotherapy may be safe to administer at higher than the traditional 2.0 mg/m2 dosage for dogs with MCT. This dose escalation may result in improved response rates when compared to the standard 2 mg/m2 dosage; randomized prospective trials will be required to establish this.

Estimation of lung lesion burden by MRI and stereology in guinea pigs experimentally infected with M. tuberculosis.

E Eschelbach, S Kraft, C Shanley, E Smith, J Troudt, A Izzo, I Orme, R Basaraba

The development of foci of granulomatous inflammation in the lung is the most common clinical manifestation of naturally occurring tuberculosis in humans and in laboratory animals experimentally infected via aerosol with M. tuberculosis. Since lung lesion size correlates with virulence of M. tuberculosis and reflects resistance conferred by vaccination, we investigated methods to estimate lung lesion burden that are rapid, sensitive and could be used anti-mortem to monitor the progression of disease or the efficacy of newly developed anti-tuberculosis drugs or vaccines. Guinea pigs were either sham-vaccinated with saline or with M. bovis BCG (BCG) 4 weeks prior to aerosol infection with the H37Rv strain of M. tuberculosis. Following euthanasia at various time points post-infection, lungs were perfusion fixed, removed en-block and subjected to magnetic resonance imaging (MRI). Total lung volume and lung lesion volume were measured from image stacks by stereological analysis and compared to measurements taken from paraffin embedded or frozen histologic sections of the same lungs. Absolute lung and lesion volumes obtained from MRI sections were consistently higher than both paraffin embedded and frozen sections which reflected shrinkage (28% frozen) and (44% paraffin embedded) associated with tissue processing. However, when expressed as a ratio of total lesion/lung volume, values were comparable. Reduction in lesion volume in BCG-vaccinated animals was reflected by a significant decrease in absolute lesion volume and a decrease in lesion/lung volume ratio (non-vacc. MRI 1.73 cm^3, non-vacc. histo. 0.83 cm^3, BCG MRI 0.56 cm^3, BCG histo. 0.20 cm^3) 30 days post infection. These data show that stereological estimation of lung lesion volume based on MRI images are feasible to be developed as method to estimate lesion burden anti-mortem.

Investigating the Efficacy and Toxicity of Two Tirapazamine Analogues in a Nude Mouse Model

M.J. Dorie, J.M. Brown, D.M. Bouley

The aberrant vasculature that is characteristic of solid tumors produces areas of hypoxia. Hypoxic cells are frequently resistant to both radiation therapy and chemotherapy. A new trend in anti-cancer therapy is to exploit this unique feature of the tumor microenvironment through the use of selective hypoxic cell toxins. The bioreductive drug tirapazamine (SR4233) is a prototypical hypoxic cytotoxin that has been associated with bone marrow suppression and retinal toxicity, limiting the drug's clinical usefulness. Purpose: This project evaluated the efficacy and toxicity of two tirapazamine analogs (SN29751 and SN30000). Methods: To assess the efficacy of the two analogues, each of 56 athymic nude mice received an intradermal injection of SiHa (a human cervical tumor cell line) cells on its dorsum. Mice were separated into treatment groups and the four-day treatment regimen commenced. The analogues were given alone at 75% of their LD50 in addition to various concentrations in combination with 8 fraction local irradiation. Ellipsoid tumor volume was plotted against time to produce a re-growth delay curve. To evaluate the toxicity of the two analogues, a toxicology assay was performed using 30 female Charles River (nu/nu) nude mice. To each group (n=10) either SN29751, SN30000, or saline was administered via i.p injection twice daily for four consecutive days. On days 12 and 22, five mice from each group were assayed. Each assay included a CBC and chemistry panel , a gross necropsy and histological analysis. Results: Tumor regression data indicated that both of the analogs produce regression curves similar to tirapazamine. The toxicology assay revealed that SN29751 produced hepatic and cardiac necrosis. Conclusions: The toxicology and tumor regression data indicate that SN30000 shows a great deal of promise as a new hypoxic cytotoxin, as it demonstrates similar efficacy to tirapazamine with reduced toxicity.

Epidermal Growth Factor Promotes the Malignant Phenotype in Canine Hemangiosarcoma

KC McDermott, BJ Rose, DH Thamm.

Purpose: Hemangiosarcoma (HSA) is one of the most common neoplasms in dogs and is often very aggressive in behavior. The receptor tyrosine kinase EGFR (erbb1), the receptor for epidermal growth factor (EGF) and other related growth factors, mediates a variety of oncogenic functions in human epithelial neoplasms. While previous studies have demonstrated the presence of EGFR in canine neoplastic tissues, the functional role played by signaling through this receptor has been incompletely studied, and its presence in HSA has not been evaluated. The primary goal of this study was to determine the in vitro effects of EGF on the proliferation, invasion, survival, and chemosensitivity of canine HSA cells, as well as our ability to block those effects using the investigational small molecule ZD6474. Materials/methods: HSA cell lines used were DEN-HSA and Fitz, both developed in our lab. EGF-driven growth alterations were measured via a bioreductive fluorometric assay (Alamar BlueTM). VEGF production was measured by ELISA and cell migration assays were performed using Boyden chamber assays. Anchorage-independent growth was evaluated using culture plates coated with poly-HEMA. Results: Under low serum conditions, both canine HSA cell lines proliferated and showed enhanced chemotaxis in response to rhEGF. Both of these responses were blocked by the addition of ZD6474. VEGF production by the cells was stimulated by the addition of EGF, and this response was blocked using ZD6474. Chemosensitivity to doxorubicin in the presence of EGF increased as ZD6474 was added. The presence of EGF promoted anchorage-independent growth; this effect was blocked by ZD6474. Studies are ongoing to evaluate phosphorylation of downstream targets in response to stimulation with EGF. Conclusions: EGF was shown to stimulate multiple features promoting the malignant phenotype in canine HSA. Strategies targeting EGFR signaling may hold promise as novel treatments for this common canine cancer.

Detection of canine distemper virus in canine blood samples by real-time reverse transcription polymerase chain reaction.

B Podell, K Pabilonia, C Duncan, C Gerhard, H Van Campen

Purpose: Development of a real-time reverse transcription polymerase chain reaction (RT-PCR) assay for detection of canine distemper virus (CDV) that is suitable for use as a diagnostic testing method. Materials and Methods: A real-time RT-PCR assay using a dual-labeled fluorogenic probe on a Smart Cycler platform was developed for detection of CDV in canine blood samples. The primers and probe were designed to target the conserved central region of the CDV nucleoprotein gene using Primer Express software and sequence information available on GenBank. A total of 330 diagnostic canine blood samples were used to validate the assay in a diagnostic setting by comparing results of the real-time RT-PCR assay to a conventional RT-PCR assay. Results: The standard curve produced for the real-time RT-PCR assay showed an amplification efficiency of 96.2%, and the minimum detection limit of the assay was 50 copies of viral RNA. Comparison of the real-time RT-PCR assay to the conventional RT-PCR assay provided comparable results. 108 out of 110 samples that were positive for CDV by conventional RT-PCR were also detected as positive by real time RT-PCR. 211 out of 220 samples that were negative for CDV by conventional RT-PCR were also found to be negative by real time RT-PCR. Conclusions: Investigation of the contradicting results between real-time RT-PCR and conventional RT-PCR assays indicated that both negative and positive results were obtained incorrectly by the conventional RT-PCR assay. The standard curve analysis along with genetic sequencing indicated that the real-time RT-PCR assay is both highly sensitive and specific. Based on our findings, we can conclude that the real-time RT-PCR assay is suitable for use in a diagnostic setting.

Biosecurity Programs at American Veterinary Medical Association accredited Veterinary Teaching Hospitals

KM Benedict, PS Morley, DC Van Metre, AA Ruple

Optimizing infection control and biosecurity is a vital part of delivering the highest quality veterinary medicine to patients and clients. Though veterinary teaching hospitals (VTHs) today typically believe in the value of infection control practices, there are no published studies characterizing components of these infection control programs. Therefore, the objective of this study was to characterize the Biosecurity practices at American Veterinary Medical Association (AVMA) accredited VTHs. The Hospital Directors at all 38 AVMA accredited VTHs were asked to identify the person most knowledgeable about the Biosecurity and infection control practices at their institution. In some situations, the Biosecurity expert was the Hospital Director, but other experts identified included Biosecurity Directors, Chairs of Infection Control Committees, or Infection Control Officers. The identified expert was invited to participate in a 15-20 minute, voluntary and confidential phone interview which was designed to be brief, but focused on the main principles of an optimal biosecurity foundation. The interview included about 60 questions on the topics of hygiene, surveillance, patient contact, education/awareness, program structure, and biosecurity expert opinion. Results indicated that most VTHs had Biosecurity programs documented as a written policy that was implemented and supported by an infection control committee. Hygiene protocols and surveillance activities varied amongst VTHs and were more stringent after having outbreaks of nosocomial infections within the past five years. The perception of program rigor and rank in comparison to other AVMA VTHs was difficult for participants to grasp since little information has been available in the past. Summarizing the status of Biosecurity programs at AVMA accredited institutions will help hospital administrators to better optimize patient care and infection control at their hospitals.

Evidence that elevated Hematocrit and Atrial Natriuretic Peptide are early markers for Cattle at Risk for High Mountain Disease.

B Fraser, T Holt , A Hill, E Swenson, P Bartsch, M Gassmann, M Tissot van Patot.

Bovine high mountain disease (HMD) or ‘brisket disease’ occurs in cattle exposed to elevations above 2000 m. HMD is characterized by elevated pulmonary artery pressure (PAP) leading to right heart failure and brisket edema. As HMD can affect 20 – 80% of a single herd, this disease causes catastrophic economic loss to ranches. To identify cattle at risk, PAP is currently measured in cattle at altitudes above 2000 m, a long and arduous process. Animals showing a PAP higher than 50 mmHg are transferred below 2000 m and sold for slaughter. Our goal is to identify a circulating biomarker of pulmonary hypertension and/or cardiac distress that correlates with elevated PAP thereby allowing early disease detection with a less invasive method. Methods: PAP was determined via jugular venous catheter in 40 Angus bulls at 2500 m. Plasma was obtained from 20 cattle with low and 20 with high PAP. Enzyme-linked immunoassays were used to detect brain natriuretic peptide (BNP), troponin, atrial natriuretic peptide (ANP) and endothelin-1 (ET-1). Radio-immunoassay was used to determined erythropoietin (EPO). Hematocrit and coagulation parameters pro-thrombin time (PT), partial thromboplastin time (PTT), and fibrinogen were determined using standard methodology. Results. Bovine BNP was not detectable by antibodies available and troponin, ET-1, PT, PTT and fibrinogen were not different between animals with low and high PAP. ANP and hematocrit increased in direct correlation with elevated mean and systolic PAP. EPO was not increased in correlation with PAP, but was 2-3 fold higher in animals with clinical HMD symptoms. Conclusions. We postulate that circulating ANP and high hematocrit are markers for animals at risk for developing HMD, while elevated plasma EPO occurs in animals with manifest HMD. Funding: Anesthesiology, UCDHSC.

Use of placental tissue for diagnosis of bovine viral diarrhea virus persistently infected alpaca crias

A Kean, R Callan

Introduction: Bovine viral diarrhea virus (BVDV) has recently become a disease of concern for alpaca producers. Like calves, alpaca crias are capable of becoming persistently infected (PI) and will efficiently shed virus for the rest of their life. BVDV is a costly disease in both cattle and camelid livestock industries. Testing for BVDV PI status is critical in the alpaca industry to identify persistently infected animals and remove them from the herd. Currently, testing is available using blood samples which in many cases require a veterinarian to obtain. We propose that placenta tissue obtained near the time of birth of the cria can be used to determine the BVDV infection status of the cria. The placenta sample can be collected by the owner and submitted quickly for reverse transcriptase PCR as an alternative to blood sampling. Objective: The objective of this study was to determine if placenta tissue could be used to identify BVDV infection in newborn alpaca crias. Procedures: Placenta tissue and whole blood samples were collected from 20 newborn crias within 24 hours of birth. BVDV RT-PCR was performed on both the placenta and blood samples and results were compared. Results: Of the 20 crias sampled, 2 were positive on both placenta and blood sample and 17 were negative for both samples. One animal tested positive on placenta but negative on blood. BVDV PCR of placental tissue samples showed a sensitivity of 100% and a specificity of 94.4% when compared to whole blood PCR. Conclusions and Clinical Relevance: We conclude that BVDV PCR of placenta tissue is a good screening test that allows for quick determination of BVDV negative crias. Whole blood PCR should be performed on animals with a positive placenta PCR test in order to confirm BVDV persistent infection . The ease of collecting placenta tissue makes this a useful tool for alpaca producers to screen their crias, reduce isolation time for the dam and cria, and minimize contamination of their premises.

Poster Presentations ~ Oklahoma Room

Post-Doc: Basic Science

Graduate Students: Basic Science

Graduate Students: Clinical Science

PVM: Basic Science

PVM: Clinical Science

Faculty

Protection of Mouse Bone Marrow Cells by Resveratrol against Radiation-Induced Chromosome Damage

R Carsten, R Ullrich

The aim of this study was to determine if resveratrol protects bone marrow cell chromosomes from radiation-induced damage. Resveratrol has been shown to have antioxidant properties, contribute to cell cycle arrest, and induce apoptosis in damaged cells. The protective effects of resveratrol were evaluated using ten week old, male CBA/CaJ mice that were placed into four groups for each time point (1 and 30 days). The groups of 10 mice consisted of the following: 1. no treatment, 2. resveratrol only, 3. radiation only, and 4. resveratrol and radiation. Resveratrol was given by gavage at 100 mg/kg once per day for two days prior to irradiation, on the day of irradiation, and then continued mixed in the water. Irradiated mice received 3 Gy whole body radiation. At each time point, bone marrow cells were collected, processed, and placed onto microscope slides. Giemsa stained slides were blinded and 25 cells from each mouse were scored for chromosome aberrations. Administration of resveratrol prior to whole body irradiation had a significant impact on the number of chromosome aberrations observed at both time points. One day following whole body irradiation, a low average of chromosome aberrations per cell were seen in the no treatment (0.17) and resveratrol only (0.17) groups while 2.60 and 1.38 chromosome aberrations per metaphase cell were observed in the bone marrow cells of the radiation only group and the radiation and resveratrol group respectively. At 30 days, a low average of chromosome aberrations per cell were seen in the no treatment (0.06) and resveratrol (0.05) only groups while 0.54 and 0.18 chromosome aberrations per metaphase cell were observed in the bone marrow cells of the radiation only group and the radiation and resveratrol group respectively. These findings indicate that resveratrol administration at 100 mg/kg, initiated prior to whole body radiation has protective effects against radiation-induced chromosome damage in bone marrow cells.

Membrane Impermeable Estradiol-Induced Increase in Number of Gonadotropin-Releasing Hormone Receptors in Cultured Ovine Pituitary Cells

TL Davis, CM Clay, TM Net

That estradiol (E2) increases the number of GnRH receptors (GnRHR) on gonadotrope cells is well established. Estradiol-induced increase in the number of GnRHR requires both mRNA transcription and protein synthesis. Despite the central role of E2 in regulation of the GnRHR gene, the underlying mechanisms are unknown. Classically at the target tissue, E2 crosses the plasma membrane of the cell, binds to the ER within the cytoplasm and translocates to the nucleus. Ligand-bound nuclear receptors bind as homodimers or heterodimers to an estrogen response element (ERE) on the promoter of target genes. However, an ERE has yet to be identified in GnRHR gene in any species studied to date. Recently, our laboratory has demonstrated effects of E2 on pituitary function that are presumably mediated at the plasma membrane. Based on the lack of an identifiable ERE in the GnRHR gene and membrane effects of E2, we hypothesized that E2 stimulates an increase in number of GnRHR by a membrane mediated mechanism. To test this hypothesis, dissociated ovine pituitary cells were treated with various doses of E2 or membrane impermeable E2 (E2-BSA; 0.1 pM - 10 nM) for 12 h. Maximal increases in the number of GnRHR occurred at 0.1 nM E2 and 10 nM E2-BSA. We then tested if the time at which maximum GnRH analog binding occurred was similar between E2 and E2-BSA. Binding was examined after cells were treated with E2 (0.1 nM) or E2-BSA (10 nM) for 3, 6, 9 and 12 h. Both, E2 and E2-BSA induced an increase in GnRH analog binding above controls at 6, 9, and 12 h. Estradiol and E2-BSA-induced increase in number of GnRHR was inhibited by the ER antagonist ICI 182,780. To establish that the effect of E2-BSA on number of GnRHR was mediated via the interaction with membrane E2 receptors and not via nuclear E2 receptors, cells were treated with E2-BSA FITC (10 nM) to confirm that E2-BSA did not translocate to the nucleus. Following 3, 6, 9 and 12 h of incubation with E2-BSA FITC, no nuclear staining was observed by confocal microscopy in the pituitary cells. We conclude that E2-BSA stimulated an increase in the number of GnRHR comparable to E2 and that stimulation of GnRHR was mediated by a membrane action. Supported by USDA-NRI 2005-35203-15376 and NIH training grant T32 HD07031-29

La Crosse Virus Vaccine and Immunotherapy: VLPs and CLDCs

MT Hughes, E Arthun, K Pandher, N Marlenee, S Mejia, C Blair, R Bowen, S Dow, BJ Beaty, R Titus

Despite the significance of bunyaviruses to public and veterinary health, their potential to emerge in new areas, and their significant potential for use as bioterrotism agents, little is known concerning the pathogenesis of these viruses in mammalian hosts, the host innate and acquired immune responses following aerosol and vector-transmission, and a lack of suitable vaccines for most viruses in this family. In this study, we determined the aerosol infectivity of La Crosse virus (LACV) in the mouse model. Additionally, we began studies using cationic lipid/ DNA complexes (CLDCs) as an immunotherapy for protection of mice exposed to LACV in this fashion. We have further begun studies to produce virus-like particles for LACV which we will then use as immunogens for LACV vaccination studies in mice.

The role of Nucleophosmin/B23 as a regulator of mRNA metabolism

H Ibrahim, AMorrison, V Palaniswamy, CJ Wilusz and J Wilusz

Purpose: Our goal was to identify proteins deposited on mRNA as a result of the polyadenylation process and characterize their role in mRNA metabolism. Materials/Methods: We utilized HeLa cell nuclear extracts to perform in vitro polyadenylation assays and UV cross-linking to visualize proteins bound to the substrate RNAs. Mass spectrometry was employed to identify proteins of interest. Knockdown experiments using siRNA as well as tethering of proteins to reporter genes are currently being used to probe the role of identified proteins in post-transcriptional processes like pre-mRNA splicing and/or mRNA turnover. Background: Nucleophosmin/B23 (NPM) is a multifunctional phosphoprotein that has been implicated in ribosome biosynthesis, p53 regulation, nuclear-cytoplasmic shuttling, and centrosome duplication. Furthermore, NPM has been associated with cellular proliferation and cancer. Results: Our results have shown that NPM specifically associates with mRNAs as a result of 3'-end formation in vitro. NPM deposition on the transcript is independent of cleavage (the first step of polyadenylation), but requires poly(A) addition. Moreover, we have shown that NPM is bound to poly(A)+ mRNAs in living cells. Hypothesis: We hypothesize that NPM is deposited on mRNAs as a marker for successful polyadenylation so it may affect downstream events associated with gene expression like splicing and export. Experiments are under way to characterize the requirements for NPM binding. We are also testing the role of NPM in the splicing and export of mRNA. Finally, we will be using microarray profiling to analyze the array of transcripts associated with NPM under various cellular conditions. Conclusions: Nucleophosmin binds to mRNAs in cells as a result of the polyadenylation event. This novel property may explain some of the roles nucleophosmin plays in RNA transcription, metabolism, and/or processing.

Light-Evoked Responses of Cultured Melanopsin-Expressing Retinal Ganglion Cells

ATE Hartwick, J Yu, KT Stevens, WH Baldridge, PJ Sollars, and GE Pickard

Purpose: A small subset (< 2%) of mammalian retinal ganglion cells express the photopigment melanopsin and are intrinsically photosensitive (ipRGCs). We have developed a novel approach for the study of these rare photoreceptive neurons by preparing enriched cultures of rat ipRGCs using anti-melanopsin-mediated immunopanning. Methods: Following the dissociation of retinas from 6-7 day old rats, a two-step panning method was employed using an antibody directed against the N-terminus of melanopsin. After 1-3 days in culture, cells were exposed to broad-spectrum illumination (halogen light source) and light-evoked responses were monitored using fura-2 calcium imaging and perforated patch-clamp techniques. Results: The cell cultures were highly enriched with melanopsin RGCs as determined by immunohistochemistry using anti-rat melanopsin C-terminus antibodies and by RT-PCR. In response to light stimulation, the isolated cells exhibited increased intracellular calcium levels. Light-induced depolarization and increased firing in the melanopsin-panned cells were also observed with the patch-clamp recordings, performed in current clamp mode. Application of the compounds 2-APB (100 μM; n=11), SKF 96365 (25 μM; n=12), La3+ (10 μM; n=7), Gd3+ (10 μM; n=7) and flufenamic acid (100 μM; n=6) reversibly blocked (mean block > 80% for all compounds; $P < 0.01$ as compared to initial light responses with drug absent) the light-evoked Ca2+ responses. The effect of these compounds is consistent with the involvement of canonical transient receptor potential (TRPC) channels, which are similar to the TRP-like channels associated with phototransduction in certain invertebrates. Conclusion: The generation of highly enriched cultures of photosensitive melanopsin RGCs, which enables the study of these photoreceptors in complete isolation, will serve as a useful system for the further characterization of melanopsin-related light signaling pathways.

Simultaneous versus alternate tensioning of wires in a single ring fixator construct

SD Ryan, N Ehrhart, K,Zuehlsdorff, S James.

PURPOSE: Simultaneous tensioning of circular external fixator wires is recommended to avoid unequal wire tensions and ring deformation. We hypothesized alternate (ALT) tensioning would result in unequal wire strains that would decrease construct stiffness in axial loading compared to simultaneous (SIM) tensioning. MATERIALS/METHODS: Wire strain characteristics of two 1.6mm 60o angle divergent wires (W1=below ring, W2=above ring), either ALT or SIM tensioned to 90kg in 24 single 84mm ring constructs were measured. Voltage data was collected from strain gages attached to the wires, during initial wire tensioning and bolt tightening, during cyclic axial loading between 0 and 200N (0.5 Hz) and load to wire failure at 0.5mm/s. Voltage data was converted to strain which was compared between and within ALT and SIM groups. Construct stiffness was compared between groups. RESULTS: No difference was seen in wire strains for each wire between ALT and SIM groups after tensioning nor in W1 compared to W2 strains within each tensioning method group (ALT, p=0.30, SIM p=0.58). W1 and W2 strains were not different within the ALT W1-1 group (W1 tensioned first), whereas, W1 strain was lower than W2 strain in the ALT W2-1 group (W2 tensioned first), (p=0.02). Loss of wire strain was noted with bolt tightening in all groups. As the second wire was tensioned in the ALT group, strain in the first tensioned wire increased. Decrease in wire strain (3-4.8%) after cyclic loading was seen for wires in both groups. There was no difference in construct stiffness during axial loading between ALT (234.9±5.4 N/mm) and SIM (230.9±5.4 N/mm) groups (p=0.52). Mode of failure was wire breakage at the bolt-wire interface in all constructs (W1 in 11/12 ALT and W1 in 10/12 SIM). CONCLUSIONS: ALT tensioning did not produce unequal wire strains and did not decrease construct stiffness under axial loading compared to SIM wire tensioning. With ALT tensioning, we recommend tensioning the lower wire first.

Characterization of Antigen Presenting Cells for West Nile Virus Specific T cell Subtypes

T Welte, C Ndaluka, E Powers, S Wang, J Madri, A Barrett, KE Olson, T Wang.

West Nile virus (WNV) causes a severe central nervous system (CNS) infection in humans, primarily in the elderly and immunocompromised. The murine model is an effective in vivo experimental model to investigate viral pathogenesis and the host immunity in humans. CD4+ T cells provide help for antibody responses and sustain WNV-specific CD8+ T cell responses in the CNS that enable viral clearance. CD8+ T cell responses are critical in clearing WNV infection from tissues and preventing viral persistence. Moreover, both T cell subtypes can provide host with long lasting protective effect by development of memory T cells. However, epitopes are not currently available to define WNV specific T cell responses. In this study, we developed two antigen presenting cells (APCs) for WNV specific T cell subtypes. H36.12j is a macrophage cell line initially derived from C57BL/6 mice. bEnd cell are B6 mouse brain derived endothelial cell line. The kinetics of WNV infection was characterized in both cell types by multiple methods, including real time PCR, flow cytometry analysis and indirect fluorescence staining. Based on these studies, WNV infected H36.12j cells and bEND cells were next harvested at the optimal time points and used as APCs for CD4+ and CD8+ T cells. Our results have shown that H36.12j cells can be used as APCs to define both CD4+ and CD8+ memory T-cell responses, whereas bEND cells are potent APCs for WNV CD8+ T cells only. Application of this method will be useful to elucidate factors affecting CD4+ and CD8+ memory T-cell formation.

Altered expression of CUG-BP in myotonic dystrophy leads to aberrant mRNA decay

L Zhang, J Wilusz & CJ Wilusz

Purpose: Our goal was to test whether altered expression of the CUG-BP RNA binding protein can result in changes in decay of specific mRNAs, namely TNF alpha (TNF) and IL6. As CUG-BP expression is defective in myotonic dystrophy (DM) patients, and both TNF and IL6 cytokines are elevated, our results may give insight into the pathogenesis of this disease. Methods: RNA decay and UV cross-linking analyses were used to examine the function of CUG-BP in extracts. RNA interference was used to reduce expression of CUG-BP in C2C12 myoblasts. To measure mRNA decay rates, transcription was inhibited with actinomycin D following induction of TNF and IL6 expression by addition of LPS. Abundance of TNF and IL6 mRNA was measured by qRT-PCR. Results: CUG-BP has been implicated in altered splicing and translation of transcripts relevant to DM pathogenesis. The role of CUG-BP in mRNA decay in DM has not been investigated. Our results indicate that CUG-BP enhances the rate of deadenylation of a reporter bearing the 3'UTR of TNF in vitro through direct interaction with a deadenylase. We used C2C12 myoblast cells as a model to study the role of CUG-BP in modulating TNF and IL6 mRNA decay in living cells. First, we measured the half-life of TNF and IL6 transcripts and found them both to be very unstable, decaying with half lives of ~20 min. However following depletion of CUG-BP by RNAi, abundance of both TNF and IL6 cytokines was significantly increased. We are currently examining whether this reflects an increase in half life. Conclusions: CUG-BP binds to the 3'UTR of TNF and recruits a deadenylase. This could explain the rapid decay of TNF and IL6 mRNAs in C2C12 cells. Reduction in cytoplasmic levels of CUG-BP, mimicking the effect seen in DM, led to an increase in the abundance of TNF and IL6 transcripts. This is likely due to increase stability of these mRNAs in the absence of CUG-BP. This may explain the elevated levels of these cytokines observed in DM patients.

GRADUATE STUDENT: BASIC SCIENCE

Activation of Pulmonary Innate Immunity by Liposome-DNA Complexes provides protection against Burkholderia mallei

A Goodyear, C Bosio, H Schweizer, S Dow

Background. Liposome-DNA complexes (LDC) are potent activators of innate immunity. Ligation of TLR-9 by LDC results in potent antitumor activity mediated by NK cells and IFN-g release. LDC are also capable of eliciting protective immunity against certain viral and bacterial pathogens. A recent publication has illustrated that CpG DNA can protect against a low dose Burkholderia mallei infection. Because LDC and CpG DNA stimulate similar immune responses we investigated the ability of LDC to protect against a lethal B. mallei infection. Methods. Mouse survival was used to access the ability of LDC to protect against a B. mallei infection. Pathology and bacterial burden in lung, liver and spleen were assessed by histology and bacterial culture of organ homogenate. Cell types and cytokines known to be stimulated by LDC were investigated through antibody depletion or KO mice. Results. Intraperitoneal administration of LDC resulted in increased survival of mice intranasally (IN) infected with a lethal dose of B. mallei. Pathology and bacterial burden in the organs of LDC treated mice were reduced when compared to controls. Natural Killer (NK) cell, dendritic cell (DC) and IFN-g stimulation by LDC all function in protection against B. mallei infection. Conclusions. The activation of innate immunity by LDC results in protection against IN delivered B. mallei. Uptake of LDC by antigen presenting cells stimulates IFN-g production by NK cells resulting in the activation of pulmonary innate immunity and protection against B. mallei. With no vaccine currently available for B. mallei and high levels of antibiotic resistance, LDC provide an attractive therapeutic option.

Protective efficacy of sub-unit vaccines against aerosol challenge with Francisella tularensis Schu4.

EE Kampf, KG White, JT Belisle, CM Bosio

Currently, there are no approved vaccines to prevent pneumonic tularemia. Although the Live Vaccine Strain (LVS) has been shown to protect rodents, non-human primates, and humans, the protection is typically incomplete. Furthermore, LVS has shown variability in attenuation and loss of protection upon repeated passage. Sub-unit vaccines represent an attractive alternative to live vaccines. We tested the immunogenic potential and protective efficacy of LVS sub-cellular fractions as a first step in designing a novel sub-unit vaccine for pneumonic tularemia. In contrast to live LVS, both membrane and soluble fractions induced secretion of TNF-α and IL-12, but not IL-10 from dendritic cells as determined by ELISA. The fractions were delivered intranasally (IN) and subcutaneously (SC) to mice with cationic lipid DNA complex as the adjuvant. When administered in vivo, both fractions elicited systemic IgA and IgG in the serum, but only LVS IN elicited local pulmonary IgA in the bronchial alveolar lavage. Furthermore, when fractions were delivered SC, the IgG response was higher than LVS for both membrane and cytosolic fractions. Both LVS-vaccinated mice and SC-vaccinated mice had IFN-γ producing T cells in both the lung and spleen to membrane and cytosolic fractions. LVS IN can extend the mean time to death in C57BL/6 mice and offer protection in BALB/c mice following a low dose aerosol challenge with Schu4. LVS sub-cellular fractions significantly extended the mean time to death when delivered IN and SC. In C57BL/6 mice, LVS extended the mean time to death, but only the soluble fraction delivered SC offered 20% protection. In BALB/c mice, LVS afforded 60% protection while the soluble and membrane fractions afforded 20% and 10% protection likewise. This data suggests that sub-unit vaccines may be capable of providing protection against pneumonic tularemia and that they are viable alternatives to the use of a live attenuated vaccine such as LVS.

In Vitro Model of Pneumonic Burkholderia Infection and Response to Combined Antimicrobial and Immunotherapy

LM Kellihan, H Schweizer, and SW Dow.

Purpose: Burkholderia mallei is a zoonotic bacterial pathogen, causing glanders in horses and systemic and pulmonary infections in humans. Little is known about the initial target cells for Burkholderia infection in the lungs or the cellular response to infection. Also, Burkholderia infections are difficult to treat due to broad antibiotic resistance. Thus, chronic infection can occur, often causing severe disease. Immunotherapy using liposome-DNA complexes elicits cytokines important in fighting bacterial infections, such as IFN-g and TNF-a. Since neither antimicrobials nor immunotherapy is capable of eliminating Burkholderia infection alone, we propose that combination treatment may produce a synergistic effect that eradicates Burkholderia infection. Materials/Methods: An in vitro infection model was developed using attenuated B. thailandensis. Three pulmonary cell lines (airway epithelial cells, pulmonary capillary endothelial cells and alveolar macrophages (AM)) were used to assess the ability of B. thailandensis to infect, replicate within and kill lung cells. Also, we are developing an in vitro model to evaluate the effectiveness of combination antimicrobial and immunotherapy at eradicating intracellular Burkholderia. Results: B. thailandensis infected and replicated within all 3 pulmonary cell lines in vitro. Infection induced killing of AM, whereas lung epithelial and endothelial cells survived. Preliminary data suggest that ceftazidime may be the optimal antibiotic for combination therapy. The effects of combined treatment with ceftazidime, IFN-g and TNF-a are being evaluated. Conclusions: B. thailandensis appears to have broad tropism for cells in the lungs. Infection of AM elicits immune activation followed by cell death, suggesting that elimination of APCs may play a role in the pathogenesis of pneumonic Burkholderia infection. We hypothesize that combined antimicrobial and immunotherapy may lead to more effective eradication of Burkholderia infection.

Instability in Radiation-Induced Acute Myeloid Leukemia

P Le, RL Ullrich, SM Bailey

Acute Myeloid Leukemia (AML) is the most common type of leukemia in adults. Radiation-induced AML is a concern for space travelers and persons treated for cancer. Deletion of PU.1, a transcription factor that plays a role in hematopoiesis, has also been correlated with this disease. In murine AML, one copy of PU.1 is frequently lost, while the other suffers a point mutation. Telomeres, tandem arrays of TTAGGG repetitive sequence, function to protect and stabilize chromosome ends. Telomere dysfunction and chromosomal instability have been associated with many types of cancer. The purpose of this study is to assess telomeric and chromosomal instability in murine radiation-induced AML. In order to undergo these studies enlarged spleens were collected from AML susceptible CBA male mice exposed to varying doses of HZE (high energy, high LET) or gamma radiation (low LET), were briefly cultured, then harvested for metaphase chromosome preparations. Utilizing cytogenetic techniques and fluorescence in situ hybridization (FISH), instability was assessed. We find increased telomeric and chromosomal instability in murine radiation-induced AML. Different types and levels of instability were observed, depending on the type/quality of radiation delivered. In conclusion these trends suggest that overall instability is increased in murine radiation-induced AML; more data needs to be gathered in order to establish significance. Interestingly, cells were mostly diploid (no numerical aberrations), so instability was not due to a gain or loss of chromosomes. Rather, instability was seen as structural aberrations. Furthermore, the types of lesions seen were dependent on radiation quality. Taken together with the work of collaborators, we find that overall instability is associated with PU.1 deletion, but is independent of microsatellite instability.

Infectious Prions in the Saliva and Blood of Deer with Chronic Wasting Disease

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Purpose: A critical concern in the transmission of prion diseases, including chronic wasting disease (CWD)—a transmissible spongiform encephalopathy (TSE) of cervids—is the potential presence of prions in body fluids. Materials and Methods: To address this issue directly, we exposed cohorts of CWD-naïve, hand-raised deer to saliva, blood, or urine and feces from CWD-positive deer. The recipient animals were monitored for CWD infection under rigorous indoor isolation conditions to exclude potential adventitious prion exposure. Results: We report the presence of infectious prions capable of transmitting CWD in saliva (by the oral route) and in blood (by transfusion). Surprisingly, we were not able to detect transmission of infectious CWD in naïve recipient deer fed urine and feces from CWD-positive donors, despite multiple exposures. Conclusions: The presence of infectious prions in saliva helps to explain the efficient transmission of CWD in nature. The presence of infectious prions in the blood and saliva of CWD+ deer establishes a basis for developing antemortem detection of the disease by blood-based assay methods.

Combining Pattern Recognition Receptor Agonists for Enhanced Activation of Innate Immunity

S. McCorvey, S. Dow

Methods: In-vitro assays of innate immune activation were performed using macrophage cell lines and cultures of normal mouse spleen cells. Agonists were complexed to liposomes, then added to the cell cultures and cytokine release was quantitated by specific ELISA. We focused on release of TNF-a, IL-12, IFN-b and IFN-g as the major measures of immune activation. Liposome-PRR agonist combinations were also evaluated in vitro for induction of antitumor activity, using mouse tumor models. Results: We found that the combination of liposomes with two different Toll-like receptor (TLR) agonists (eg, DNA and poly I:C) or a TLR agonist plus a Nucleotide-binding Oligomerization domaion (NOD) receptor agonist (eg, DNA plus Muramyl-dipeptide) produced the greatest enhancement in immune activation over DNA alone. Conclusions: Certain combinations of PRR agonists, when introduced into cells using cationic liposomes, can induce synergistic immune activation. These PRR agonist combinations warrant further in-vivo evaluation as potent immuno-theraputic.

The role of D7 protein in West Nile Encephalitis

K Reagan, C Machain-Williams, R Lanciotti, J Beebe, K Olson , B Beaty, C Blair, and T Wang.

Mosquito saliva proteins are known to be involved in facilitating blood uptake and modulation of host immune response. The D7 family protein has been recognized as specifically expressed in the salivary glands of adult Diptera. The functions of D7 proteins are not known yet. Culex (Cx.) mosquitoes are the documented major vectors of West Nile virus (WNV). In previous work, we found that immunization of mice with whole salivary proteins of Cx. tarsalis (a common mosquito of WNV in the western U.S.) induced a strong Th1 type immune response, thereby limiting virus dissemination upon subsequent mosquito inoculation of WNV. Immunoblot analysis of Cx. tarsalis salivary proteins shown two prominent antigens recognized by sera against D7 protein of Aedes aegypti. Here we have focused on D7 protein of Cx. tarsalis. Initially, we collected the saliva of Cx. tarsalis by using capillary tube collection. These proteins were next precipitated using trichloroacetic acid. Further, we analyzed the reactivity of salivary proteins to WNV patient sera from an outbreak in Colorado by western blot. Two major antigenic proteins were recognized. The size of these proteins corresponds to the D7 salivary proteins found in other species. Positive bands were excised and analyzed by mass spectrometry to determine the protein sequence. To fully identify D7 gene of Cx. tarsalis, we designed degenerate primers to the Cx. tarsalis D7 gene based on the published sequence of Cx. pipiens quinquefasciatus. Total RNA was isolated from Cx. tarsalis, and cDNA to the D7 gene was made. Amplified PCR products were cloned. Clones were selected and sequenced. Nucleotide sequence matched the D7 gene of Cx. p. quinquefasciatus with a 55% homology. Overall, these data suggest a possible involvement of D7 protein in WNV induced disease. Results from this study will provide new insights of the pathogenesis of WNV encephalitis.

The Immunohistochemical Expression of PrPc in, and Experimental Transmission of Chronic Wasting Disease to, a Transgenic Mouse Model.

DM Seelig, GL Mason, GC Telling2, EA Hoover.

Chronic Wasting Disease (CWD) is a naturally occurring prion disease of cervids. In this, and other prion diseases, the normal prion protein (PrPc), is transformed from an alpha-helical rich isoform to a protease-resistant, beta-sheet rich isoform (PrPres). In our studies, we are utilizing cervid prion protein (CerPrP) expressing transgenic (Tg[CerPrP]) mice to evaluate the neuropathogenesis and clinical disease features of CWD. As part of our early work, we are presenting three pieces of data: i) the distribution of PrPc as documented by immunohistochemistry (IHC), ii) demonstration of CWD-susceptibility of Tg[CerPrP] mice following inoculation via intracerebral (IC), intraperitoneal (IP), and intravenous (IV) routes, and iii) early evidence for a paradoxical syndrome of weight gain in CWD-infected mice.Using a combination of perfusion fixation with paraformaldehyde-lysine-periodate (PLP) fixative and the anti-PrP antibody R505.5, PrPc immunoreactivity of variable intensity was identified in a wide variety of tissues, including those of the nervous, lymphoid, gastrointestinal, and endocrine systems. Such immunoreactivity was not observed in PrP knock-out mice and could be effectively abrogated following a dual-step, formic acid immersion protocol. Moreover, at 60, 90, and 120 dpi, we utilized IHC to demonstrate PrPres in tissues of IC, IV, and IP, but not PO inoculated mice. Finally, similar to results obtained from our ongoing CWD studies in white-tailed deer, we have observed an unexpected syndrome of weight gain in infected Tg(CerPrP) mice. This gain precedes neurological symptoms and presages evidence of wasting. These results provide data necessary to support the role of this transgenic mouse construct as a model of CWD infection and for the study of the mechanisms of this previously unreported metabolic syndrome.

Bovine Viral Diarrhea Virus Infection During Fetal Development

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Purpose: Noncytopathic Bovine Viral Diarrhea Virus (BVDV) infection during early gestation (d.150 or postnatal) results in a transient infection that is resolved through clearance of the virus and seroconversion. BVDV infection of pregnant heifers at two stages of gestation was studied to identify differences in the innate immune response and the effects on fetal growth and tissue pathologies. Materials/Methods: BVDV naive pregnant heifers were infected with ncp-BVDV type 2 on d. 75 (n=6) or d. 175 (n=6) of gestation. A control group (n=6) was maintained free of BVDV. Fetuses were collected by caesarean section on d. 190. Infection was confirmed by antigen-capture ELISA and qtRT-PCR, and fetal gross anatomical and histological analyses were performed. Spleen and liver samples were analyzed by qtRT-PCR for BVDV RNA and expression of ISG15, a marker of Type-1 interferon (IFN) production. Results: Fetuses infected on d.75 presented with inhibited growth and pathologies of several tissues, including liver, bone, and heart. High BVDV RNA levels were detected, accompanied by low expression of ISG15, indicating a mild IFN response. Fetuses infected later (d. 175) developed normally, and displayed dramatic upregulation of ISG15 and a low amount of BVDV RNA. Conclusions: BVDV infection of bovine fetuses during early gestation results in a persisting viremia that invokes a weak IFN response and adverse effects on fetal growth and development. However infection during later gestation results in a strong IFN response and a controlled infection.

Multiple sequence elements within the VEE 3'UTR repress poly(A) shortening.

KJ Sokoloski, CJ Wilusz, J Wilusz.

Purpose: Alphavirus genomic and subgenomic RNAs strongly resemble cellular mRNA in that they possess a 5'cap structure and a 3'poly(A) tail. The organization of the Alphavirus 3'UnTranslated Region (UTR) consists of a Conserved Sequence Element (CSE), a U-Rich Region (URR) and a set of Repeat Sequence Elements (RSEs.) While the CSE is essential for viral replication, the URR and RSEs have no described function. The goal of this study is to determine if elements present in the alphavirus 3'UTR are capable of protecting an RNA substrate from decay. Eukaryotic cells possess specialized RNA decay machinery capable of robustly and specifically removing unwanted mRNAs from their cytoplasm. Generally, degradation of host mRNA occurs by deadenylation of the RNA followed either by 3' to 5' degradation, or by 5' to 3' decay initiated by removal of the 5' methyl-guanosine cap. Materials and Methods: A reporter RNA containing the 3'UTR of an alphavirus, Venezuelan Equine Encephalitis Virus (VEE), was assayed for resistance to decay using a mosquito cytoplasmic extract. Substrates were incubated in the presence of extract and exposed to UV irradiation to characterize possible trans-acting factors. Results: The 3'UTR of VEE was capable of repressing deadenylation of the reporter transcript, thereby preventing subsequent 3' to 5' degradation of the body. Mutational analysis of the VEE 3'UTR, has revealed both the RSEs and the URR are able to act independently to prevent poly(A) shortening. Using cross-linking analysis, a 40kD factor whose binding correlates with inhibition can be observed. Conclusions: The 3'UTR of VEE is capable of inhibiting deadenylation via interaction with a cellular trans-acting factor. The RSEs of VEE, which previously had no known function, are indeed RNA stability elements as we hypothesized. Furthermore, this function is evolutionarily conserved in other alphaviruses.

Using Chimeric FIV Constructs to Assess Virulence Determinants.

J Thompson, J Gruber, K Randers, E McNulty, DS Stump, S de Rozieres, JH Elder, and S VandeWoude.

Purpose: Relatively minor variations in lentiviral genotype can result in substantial differences in pathogenicity to the host. Feline immunodeficiency virus (FIV) is a naturally occurring immunodeficiency-inducing lentivirus of cats that provides a useful animal model to study the mechanisms of this phenomenon. For instance, while infection with the clade A isolate FIV-PPR results in a disease course marked by acute viremia followed by a long asymptomatic phase, infection with the clade C isolate FIV-C-PG leads to rapid immunodeficiency marked by CD4+ T cell depletion, malaise, and opportunistic infection in domestic cats. Materials/methods: To test the hypothesis that the envelope gene is a determinant of viral pathogenesis, a chimeric virus, FIV-C.Env, was constructed by inserting the 3' region of FIV-C-PG, including Vif, Orf A, Env, and Rev1, into the FIV-PPR background. An additional chimera, FIV-C.3'LTR, containing the 3' long-terminal-repeat of FIV-C-PG in the FIV-PPR background was also constructed. To determine the pathogenicity of these chimeras in vivo, groups of 5 specific-pathogen-free felines were infected with FIV-PPR, FIV-C.Env, FIV-C.3'LTR, or FIV-C-PG, or sham control. Results: Infection with parental virus strains demonstrated typical virulence phenotype. FIV-C.Env was minimally infectious in vivo and displayed delayed viral kinetics relative to the parental viruses. FIV-C.3'LTR was similar to FIV-PPR in infectivity and disease expression. Conclusions: These results indicate that simple substitution of 3' elements of a phenotypically virulent FIV onto a less virulent strain does not result in altered phenotype, suggesting virus phenotype is reliant on other genotypic factors.

Specific role for hTrpC4 in signal-regulated calcium entry in the human myometrium.

A Ulloa, M Zhong, YS Kim, and BM Sanborn.

Purpose: During labor, increases in intracellular calcium ([Ca2+]i) have been closely correlated with human myometrium contractions. Extracellular calcium enters the cell through voltage-operated and signal-regulated Ca2+-entry (SRCE) mechanisms. Canonical transient receptor potential (TrpC) channels may be responsible for SRCE. Human myometrium expresses TrpC4, TrpC1 and TrpC6 mRNAs in greatest abundance relative to other TrpCs. To study specific TrpC contributions in the immortalized myometrial cell line PHM1, RNA interference (RNAi) mechanisms were utilized. Materials/methods: Designed short hairpin RNAs (shRNAs) were tested for induction of a hTrpC4 knockdown using psiCHECK-2 luciferase reporter system. Four hTrpC4-shRNA constructs effectively targeted destruction of hTrpC4-mRNA (60-90%), whereas empty vector did not induce significant changes. PHM1 cells exhibit poor transfection efficiency. Thus, adenoviral vectors were created for uniform infection with shRNAs. hTrpC4-shRNA treated PHM1 cells were analyzed for potential effects on SRCE, using Fura-2 calcium imaging techniques. Infection was verified by GFP co-expression. Results: Adenoviral constructs exhibited 90% infection efficiency. Adenovirus expressing hTrpC4-shRNA#1 induced a mRNA and protein hTrpC4 knockdown, whereas infection with empty vector had no effect. hTrpC1 and hTrpC6 mRNAs were not changed. PHM1 cells were treated with 100 nM oxytocin to test for receptor-operated SRCE and with 100 nM thapsigargin to test for store-operated SRCE. Cells infected with vector expressing hTrpC4-shRNA exhibited attenuated oxytocin-mediated SRCE, but there was no effect on thapsigargin-stimulated calcium entry. Conclusions: Adenoviral constructs expressing hTrpC4-shRNA can efficiently infect myometrial smooth muscle cells and specifically reduce hTrpC4 expression. Results implicate hTrpC4 in a specific role in receptor-operated calcium entry in PHM1 cells. Supported by NIH HD38970, T32-HD0703 and the March of Dimes.

Detection, Containment and Elimination Strategies for Mouse Parvovirus: A Contemporary Managerial Approach

G Wilkerson, ED French, D Neil, S VandeWoude

Mouse Parvovirus (MPV) continues to be a common, insidious contaminant in laboratory animal facilities worldwide. Detection and eradication of MPV has been problematic due to the poorly understood pathogenesis and environmental persistence of the virus.
Purpose: To compile contemporary information on the virus in order to better contain and eliminate an ongoing MPV infection within our centralized animal facility. The facility houses approximately 12,000 mice, including immunocompromised strains and breeding colonies, as well as other laboratory animal species. Materials and Methods: Based on our research, we implemented the following control measures: 1. Development of an intensive training program for all investigators, investigator staff and animal care staff; 2. Consolidation of all MPV-positive colonies to a single corridor; 3. Restriction of access to the effected corridor via a keycard system; 4. Adjustment of airflow within the effected mouse cages and rooms to be negative to the rest of the facility; 5. Procurement of room-specific equipment and supplies for MPV-positive rooms; 6. Adjustment of quarantine procedures for non-approved-vendor mice entering the facility to include increased time of surveillance and fecal PCR screening; 7. Establishment of quarantine and screening protocols to rescue MPV-negative colonies from MPV-positive rooms; and, 8. Intensified environmental monitoring of MVP-positive facilities in an attempt to localize sources of infection. Results: We have concluded that within our facilities MPV-positive sentinel mice do not typically represent widespread infection within rooms, that breeding colonies are at highest risk for seroconversion, and that managerial interventions can be a useful measure in the control of MPV. A 33% reduction of MPV-positive sentinels over a five-month period and containment of MPV to a few pre-chosen rooms over a nine-month time period provide evidence of this.

Sodium Valproate to Enhance Doxorubicin Sensitivity

L Wittenburg, D Thamm, L Bisson, B Rose

Purpose: Many canine cancers are incurable with current treatments, and novel forms of therapy are desperately needed. The enzyme histone deacetylase (HDAC) is a powerful new target for cancer therapy. The acetylation of histones, controlled by multiple histone acetyltransferases and HDACs is important in governing chromatin structure and thereby may modulate expression of genes associated with cellular proliferation, differentiation and survival. One available and inexpensive drug with HDAC inhibitory activity is the anticonvulsant sodium valproate (VPA). Materials/methods: Canine and human osteosarcoma (OS) cell lines were incubated with and without VPA alone and in combination with doxorubicin (DOX); the anti-proliferative effects were evaluated using a bioreductive fluorometric assay. Immunofluorescence cytochemistry and Western anaylysis were used to evaluate changes in histone acetylation. VPA effects on apoptosis were evaluated with Annexin-V staining followed by flow cytometry. Results: Treatment with VPA resulted in significant increases in acetylated histone H3 in tumor cells and canine mononuclear cells. Incubation with VPA alone had mild antiproliferative effects, while VPA/DOX co-incubation increased DOX sensitivity. Pre-incubation with VPA followed by a pulse exposure of DOX resulted in significant chemosensitization. Prolonged exposure to VPA also potentiated apoptosis induced by DOX. Conclusions: Modulation of chromatin structure can improve the anti-tumor effects of DOX in vitro. Treatment of OS cells with clinically relevant concentrations of VPA increases histone acetylation and sensitizes these cells to the antiproliferative effects of DOX. This makes VPA a useful drug to take into clinical evaluation in canine cancer patients.

GRADUATE STUDENT: CLINICAL SCIENCES

Prevalence of Rickettsia species infections in cats with and without fever

DB Bayliss, AK Miller, MC Horta, MB Labruna, JR Hawley, MM Brewer, MR Lappin.

Purpose: Recent studies have documented that cats can be infected with a number of rickettsial organisms. Antibodies against R. felis, R. rickettsii, R. akari, and R. typhi have been detected in a number of cats with and without clinical manifestations of disease. The purpose of this study was to compare the prevalence of Rickettsia sp. DNA, R. felis antibodies, and R. rickettsii antibodies in the blood and serum of cats with and without fever. Materials/Methods: Cats with a body temperature of >102.5ºF (39.2ºC) served as the fever group and age-matched cats without fever from the same clinic served as the control group. Information collected included state, age, and history of flea exposure. Rickettsia species PCR assays were performed on whole blood from 71 paired samples using oligonucleotide primers for the citrate synthase gene (gltA) and the outer membrane protein B gene (ompB). Antibodies against R. felis and R. rickettsii were detected in serum from 90 and 91 paired samples, respectively by use of IFA. Prevalence rates were compared between groups by logistic regression with significance defined as $P < 0.05$. Results: All blood samples were negative for Rickettsia DNA. Antibodies against R. felis were detected in serum from 5/90 cats with fever (5.6%), and 2/90 control cats (2.2%). Antibodies against R. rickettsii were detected in serum from 6/91 cats with fever (6.6%), and 2 control cats (2.2%). Cats with fever were more likely to be R. felis seropositive than control cats, but the results were not statistically different (P value = 0.2734). Cats with fever were more likely to be R. rickettsii seropositive than control cats, but the results were not statistically different between groups (P value = 0.1785). Conclusions: Results of this study failed to find an association between R. felis or R. rickettsii antibodies and fever. However, larger study populations will be needed to further assess the association of these organisms to clinical disease in cats.

Impact of telomerase status on canine osteosarcoma patients

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Introduction: Telomerase status is an important predictor of survival in human osteosarcoma patients. Specifically, telomerase positive patients have significantly shorter survival times compared to telomerase negative patients. It has also been shown that a majority of human osteosarcoma patients are telomerase negative (60-75%) In contrast, little is known about telomere maintenance in canine osteosarcoma. Methods: Telomeric repeat amplification (TRAP) assays were performed on thirty biopsy confirmed canine osteosarcoma tumor samples. The majority of these patients had appendicular osteosarcoma (29/30) and limb amputations. Various adjunctive treatment options were elected post operatively including some who did not receive any chemotherapy. Outcome information was obtained retrospectively from the Animal Cancer Center's canine osteosarcoma database. Results: TRAP assays revealed the presence of telomerase activity in 23/30 (76%) of canine osteosarcoma samples. The median survival of telomerase positive patients was 229 days (range: 9-858 days) compared to 209 days (range: 18-504 days) for patients who were telomerase negative. Conclusion: The majority of canine osteosarcoma patients are telomerase positive compared to human osteosarcoma patients who are predominantly telomerase negative. There does not appear to be a difference in the median survivals of canine patients based on telomerase status in this group of patients. However, this may be due to the relatively small sample size of patients who are telomerase negative. Additional statistical analysis is necessary to determine effect of other factors on telomerase and outcome.

Evaluation of Kisspeptin in the Hypothalamic Pituitary Gonadal Axis in the Mare

C Magee, JE Breummer, PM McCue, CM Clay

Kisspeptide has emerged as one of neuroendocrinology's most exciting developments in our understanding of the hypothalamic-pituitary-gonadal axis. Our current interpretation of the novel decapeptide and it's G-protein coupled receptor, GPR54, is that they are crucial elements in the onset of pubertal development and play a role in seasonal estrous and induction of ovulation via a mechanism of signaling gonadotropin release at the level of the hypothalamus. The purpose of this series of experiments was to evaluate the role of kisspeptide and GPR54 in the mare. Using rat KiSS-10, we were able to elicit a dose-dependent release of luteinizing hormone after intravenous administration of 1.0 µg, 0.5 mg, and 1.0 mg and 10.0 mg of KiSS-10. In a series of experiments to mimic the pre-ovulatory LH surge, we are in the process of measuring LH serum levels via RIA for mares given known ovulatory-inducing agents (reLH, Deslorelin). To confirm the decapeptide formulation of kisspeptin is insufficient as a single dose to induce ovulation, 1.0 mg of KiSS-10 was compared to 2,500 IU of hCG and saline. Not surprisingly, we were unable to induce ovulation in the estrus mare ($P > .05$) with kisspeptide. We have individually identified GnRH and kisspeptide neurons the equine hypothalamus via immunohistochemistry and co-localization work is ongoing. Given the homology amongst species for GPR54 and kisspeptide coding sequences, we hope to have similar success in identification of the peptide and its receptor via PCR. Although we have had some initial promising results using primer sequences designed from ovine and bovine, this work is also ongoing. In conclusion, we have demonstrated that the mare can respond to exogenous administration of kisspeptide and after further evaluation of the decapeptide and its receptor applications for induction of ovulation or seasonal transition may critically involve the use of kisspeptide.

Disease severity as a measure of M. tuberculosis virulence in the guinea pig model of tuberculosis

G Palanisamy

Virulence is the measure of pathogenicity of a microorganism as determined by the ability to invade host tissues and to produce severe disease. In the low-dose aerosol guinea pig model of tuberculosis, the virulence of multiple strains of M. tuberculosis was determined by measuring time of survival, distribution and number of culturable bacilli and the severity of pulmonary and extra-pulmonary lesions. Distribution of culturable bacilli and pathology were determined at 30 days post-infection, with pulmonary and extra-pulmonary lesion pathology scores compared at day 30 and at the time of death. Three clinical isolates had the shortest mean survival time: Erdman K01 (81 days), CSU 93/CDC1551 (87 days), HN878 (114.5 days) compared to the laboratory strain H37Rv (146.5 days). By 30 days post-infection, bacilli disseminated from lung, Erdman K01 (4.46 mean CFU), CSU 93/CDC1551 (4.15 mean CFU), HN878 (5.36 mean CFU) and H37Rv (4.83 mean CFU), resulting in microscopically visible lesions and culturable bacilli in multiple extra-pulmonary sites some of which were determined to be the cause of death. The extent of pulmonary and extra-pulmonary lesion necrosis correlated with virulence since the three clinical isolates had higher scores indicative of necrosis at 30 days post-infection and at the time of death, compared to H37Rv. The presence of pulmonary and extra-pulmonary lesions with necrosis was a better predictor of virulence than the number of bacilli (CFU) cultured from lungs, liver and spleen.

Pradofloxacin for the treatment of feline rhinitis

ME Spindel, MR Lappin, D Bachman, L Tornes

Purpose: Feline herpesvirus 1 (FHV-1), calicivirus, Mycoplasma spp., Bordetella bronchiseptica and Chlamydophila felis are the most common primary causes of upper respiratory infections (URI) in cats. While secondary bacterial infections are often successfully managed with antibiotics in the beta-lactam class, primary bacterial infections frequently are not. In these cases, administration of a tetracycline or a fluoroquinolone is generally effective. The purpose of this study was to compare amoxicillin and pradofloxacin (a new fluoroquinolone) for the treatment of suspected bacterial URI in cats residing in a humane society. Material/methods: Forty cats with URI and a suspected bacterial component were entered and randomly placed into one of three groups: amoxicillin at 22 mg/kg, PO, q12hrs for 7 days; pradofloxacin at 5 mg/kg, PO, q24hrs for 7 doses; or pradofloxacin at 10 mg/kg, PO, q24hrs for 7 doses. Prior to treatment, nasal discharges were collected for aerobic bacterial culture, anaerobic bacterial culture, aerobic antimicrobial susceptibility testing, Mycoplasma spp. culture, FHV-1 PCR assay, and calicivirus RT-PCR assay. A standardized clinical score was assigned to each cat daily by a person masked to the treatment groups. Differences in clinical scores per group and percentage responders per group were evaluated with significance defined as $P < 0.05$. Results: Drug toxicity was not noted. The most frequently isolated organisms pre-treatment were FHV-1 (75%), Mycoplasma spp. (62.5%), Bordetella spp. (47.5%), Staphylococcus spp. (12.5%) and Streptococcus spp. (10.0%). Differences in clinical scores between groups over time were not noted. Overall response rates for amoxicillin (10/15 cats; 67%), pradofloxacin at 5 mg/kg (11/13 cats; 85%), and pradofloxacin at 10 mg/kg (11/12 cats (92%) were not statistically different. Conclusions: The results suggest that pradofloxacin can be a safe and efficacious therapy for some cats with suspected bacterial URI.

Association of microalbuminuria and the urine albumin:creatinine ratio with systemic disease in cats

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The objectives of this study were to determine the prevalence of systemic disease in cats with/without MALB and the diagnostic utility of a semi-quantitative MALB kit (MALBE, E.R.D.-HealthScreen® Urine Test), quantitative MALB assay (MALBQ, ERD™ Test), urine albumin:creatinine ratio (UAC), and urine protein:creatinine ratio (UPC) for systemic disease in cats. Urine samples from 441 of 611 cats presented to Colorado State University (CSU) met inclusion and exclusion criteria. Urinalyses were performed at the CSU Clinical Pathology Laboratory. Urine dipstick (DpP; cutoff of trace), UPC (cutoffs of 0.4 and 0.1), MALBQ and MALBE (cutoffs of 1 mg/dl), and UAC values (cutoffs of 100 and 200 mg/gm) were determined. Clinical diagnoses within 3 months of urine collection were: healthy, neoplasia, infection/inflammation/immune-mediated, urinary, endocrine and other disease. The influence of clinical diagnosis, gender, age, BUN, creatinine, blood pressure, urine culture results, temperature, pyuria, hematuria and bacteriuria on MALBQ, MALBE or DpP was evaluated by logistic regression. P values < 0.05 were considered significant. All cats were positive using UPC0.1 cutoff so this test could not be further evaluated. The small number of cats positive by UPC0.4, UAC100 and UAC200 precluded their evaluation. Factors significantly associated with a positive MALBQ were health status, urinary disease, gender, BUN, pyuria, and hematuria. Factors significantly associated with a positive MALBE were urinary disease, age, BUN, creatinine, pyuria, and hematuria. The only factor significantly associated with DpP status was hematuria. Based on this study, UAC ratios were not useful for identifying underlying disease due to their poor sensitivity. In contrast, MALB is associated with the presence of disease. Further prospective studies are necessary to quantify the diagnostic utility of MALB tests for detection of occult disease in cats.

Therapeutic Targeting of Ire1 in Solid and Blood Tumors

DDK Chung, DE Feldman, AC Koong.

Hypoxia is a classical stressor that induces protein misfolding within the endoplasmic reticulum (ER) while this may also be triggered by an increased demand on the ER for protein secretion. The toxicity of misfolded proteins in the ER in solid tumors and malignant plasma cells of multiple myeloma is dealt with by a protective cell homeostatic process called the unfolded protein response (UPR). Ire1, an ER resident protein molecule, is one major pathway of the UPR that detects and regulates the accumulation of misfolded proteins in the ER. Therefore, we hypothesized that inhibition of the Ire1 pathway would hinder growth of tumor cells under hypoxia and/or ER stress. A reporter construct was transfected into HT1080, a human fibrosarcoma cell line, in which the luciferase protein would be expressed only under Ire1 activation induced by hypoxia. Also, cancer cell growth and proliferation were directly assayed when the cells of HT1080 as well as RPMI 8226, a multiple myeloma line, were incubated with or without the candidate Ire1 inhibitors in the media. Certain candidate Ire1 inhibitors showed marked decrease in luciferase activity linked to Ire1 and its downstream target XBP-1 activation when the transfected cells were stressed under hypoxia. Similar luciferase activity decrease was detected for the inhibition of branches of UPR other than Ire1. The number of surviving colonies of solid tumor cells decreased in the presence of Ire1 inhibitors in the media, but the effect was less apparent in the initial studies of the proliferation of multiple myeloma. Based upon these studies, inhibition of Ire1 and therefore one or more branches of the UPR correlates with the inhibition of growth and proliferation of solid tumor cells. Inhibition of the UPR may or may not be efficacious with multiple myeloma. Studies of in vivo efficacy of Ire1 inhibitors on solid and blood tumors will be required for the understanding of the compounds as a potential cancer treatment option.

Equine Kisspeptin and the Equine Hypothalamic-Pituitary Axis

C Corning, C Magee, T Farmerie, P McCue, and C Clay

Seasonal reproduction in horses limits lifetime fecundity of mares. Thus, development of efficacious methods to shorten seasonal anestrous remains an important goal in equine reproduction. Pituitary hormones LH and FSH drive reproductive function. When in circulation, they bind specific ovarian receptors to stimulate follicular development and ovulation. GnRH is a hypothalamic hormone that binds specific receptors on pituitary gonadotropes, stimulating synthesis and release of LH and FSH. Recently, the peptide Kisspeptin (KiSS) was found to play a key role in this axis. KiSS is produced by hypothalamic neurons in close proximity to GnRH neurons. KiSS binds a G-protein coupled receptor, KiSS1r, on GnRH neurons, leading to GnRH production and release. The goal of these studies is to determine if the genes encoding KiSS and KiSS1r are present in horses and if exogenous KiSS treatment leads to LH release in horses. To clone KiSS and KiSS1r, we aligned sequences from several species and designed oligonucleotide primers to highly conserved regions. PCR amplification from equine genomic DNA resulted in products of appropriate lengths. These will be sequenced. For the in vivo study, 6 luteal phase mares received indwelling IV catheters. KiSS was administered IV (1.0 mg or 10.0 mg) and blood samples were collected at intervals varying from 10 to 60 minutes between 2 hour before and 4 hours after injections. Mares then were given 25 micrograms GnRH as a positive control for pituitary responsiveness. Serum concentrations of LH were determined by radioimmunoassay. Two of 3 mares in both 1.0 and 10 milligram groups displayed an average 4.5 and 2-fold increase in LH respectively at 30 min following KiSS administration. Thus, KiSS stimulates LH secretion in horses and may represent a target to develop pharmacological approaches to stimulate pituitary and ovarian activity in seasonally anestrous mares.

The Use of Cancer Related Anemia as a Prognostic Indicator of Survival Outcome in a Retrospective Study of 100 Dogs with Osteosarcoma

KT Davis, V Phaybouth, C Olver

Anemia of cancer is an established concern in the treatment of human cancer patients and has been shown to significantly affect quality of life and survival outcome in these patients. Canine cancer patients often undergo similar chemotherapy treatment and may experience similar effects on quality of life and survival; however the effect of anemia in canine cancer patients is seldom explored. The scope of this research examined whether pre-treatment cancer-related anemia or sustained anemia during treatment is a prognostic factor for survival or remission time in dogs with osteosarcoma (OSA). A retrospective analysis was performed using data of 100 OSA canine patients presented to the Colorado State University Veterinary Medical Center between June 1997 and November 2002. Statistical analysis performed included Cox proportional hazards survival regression and Chi square analysis of contingency tables. Statistical analysis of pre-treatment anemic canine OSA patients revealed that there is no significant difference in remission or survival time in anemic and non-anemic OSA dogs. In addition, sustained anemia during treatment was not found to be a significant factor in determining survival or remission time. Breed, MCV values and grade of OSA were also examined as to whether they played a role in the occurrence of pretreatment anemia. Analysis showed that breed, MVC values and grade were not significant factors in the prevalence of pre-treatment anemia. Furthermore, grade of OSA was found to have no significant effect on remission time. These findings contribute to the sparse information available about pre-treatment cancer-related anemia or sustained anemia during treatment in canine cancer patients and suggest that anemia status is not a significant consideration for clinicians treating OSA chemotherapy patients. Future studies will perform the same analysis on dogs with lymphosarcoma and other tumors.

A stereological study of the uterine stroma in women with premature ovarian failure undergoing hormone replacement therapy.

S. Embrey, P. Dockery, T.C. Li and I.D. Cooke

This study examined the structure of the endometrial stroma in women with idiopathic ovarian failure in artificial cycles produced by two different types of hormone replacement therapy (oestradiol valerate orally and either intramuscular injection of progesterone or progesterone via vaginal pessary). Both of which had been claimed to be capable of supporting successful implantation. Biopsies were performed on day 19 of the artificial cycle (with informed consent and appropriate ethical committee approval). Tissues were processed for transmission electron microscopy. Semi-thin sections were cut from these blocks and examined by light microscopy. A variety of sterological probes were used to quantify the structural characteristics of these cells and the results obtained were compared with those from normal fertile subjects biopsied on day 6 after the luteinizing hormone (LH) surge. The following parameters were estimated: Volume Fraction, Volume Weighted Mean Volume and Axial Ratio.

Development of an in vitro model for assessing enterocytes and lamina propria macrophages during development of spontaneous and bacteria-induced colitis.

B Fierro, A Tolnay, H Bielefeldt-Ohmann.

Purpose: To develop an in vitro model to characterize the interactions between colonic enterocytes and enteric immune cells during the development of colitis. Methods: The in vitro model utilizes mdr1a -/- mice, deficient for a gene encoding for the multi-drug-resistant transporter P-glycoprotein, which allows bacterial toxins and antigens to pass the epithelial barrier, leading to spontaneous colitis. A model simulating the in vivo system was constructed: full-thickness samples from the large intestine were first removed for histopathology. Enterocytes and macrophages (Mo) were then isolated from the colon. Enterocytes were plated onto Matrigel-coated transwells. Mo were separated from contaminating cells by Percoll gradient centrifugation, then cocultured with enterocytes. Virulent or avirulent enteric bacteria (Enterococcus faecalis) were added either to the apical (enterocyte) chamber or to the basolateral (Mo) chamber. RNA was isolated from both cell types at varying time points and employed in microarray analysis to determine their cytokine and growth factor mRNA expression. Additionally, Mo were spotted onto slides and the expression of phenotypic markers assessed by immunohistochemistry. Results: The mdr1a-/- mice remained Helicobacter-free throughout the experimental period (6 months). Spontaneous colitis developed only in animals > 14-15 weeks of age. Prior to frank colitis there was upregulation of MHC-II antigen on enterocytes and Cox-2 in lamina propria Mo. Development of the in vitro model is complete and has successfully been used for procuring RNA samples currently being analyzed. Conclusions: Development of the in vitro model has been completed. We have successfully harvested and cocultured enteric immune cells and enterocytes, both with and without the addition of enteric bacteria. We are now focused on identifying and characterizing the key interactions between these cells leading to colitis.

The Effect of Feline Immunodeficiency Virus on CD40 Ligand Response in Bone Marrow Derived Dendritic Cells

LM Habermann, KP O'Halloran, TL Lehman, JA Campbell, SA.Fallon, PR Avery

Impairment of Dendritic cell (DC) function contributes to the pathogenesis disease. Studies examining the significance of DC dysfunction in human immunodeficiency virus (HIV) infection are ongoing. We used the feline immunodeficiency virus (FIV) model system to determine at which level DC dysfunction occurs. DCs are potent antigen-presenting cells, critical in pathogen recognition and the initiation of primary T cell response. DCs express toll like receptors (TLR) that activate the immune response via the production of cytokines in response to pathogens. We have previously shown that DCs from FIV-infected cats produce increased amounts of IL-10 relative to IL-12 in response to TLR2, TLR4 and TLR9 ligation. After initial pathogen recognition, dendritic cells amplify T cell responses through the expression of CD40, a co-stimulatory molecule that binds CD40L (CD154) on the surface of T cells. This binding results in the production of cytokines determining the efficacy of the T cell response. Dendritic cell production of IL-12 after CD40 ligation results in the expansion of CD8+ T lymphocytes, a cell-mediated response. To determine if a similar shift in the IL-12:IL-10 axis at pathogen recognition occurs at the CD40-CD40L binding, feline cytokine production was characterized after DCs were stimulated with 3T3 cells expressing CD40L. We have analyzed the cytokine response from 5 naïve and 4 FIV-infected cats and the same shift favoring IL10 production is not apparent. Instead, there is a trend to a ratio that favors IL-12 expression in the infected animals. Our results suggest that, while the initial steps in pathogen recognition are altered in FIV-infected DCs, the ability of FIV-infected dendritic cells to amplify an appropriate cell-mediated T cell response remains intact. Future studies will be directed at determining which intracellular signaling pathways are involved. This information will be useful in the design of therapies for lentiviral infections.

The Effects of Rearing Condition and Enrichment on Laboratory Mouse Immune Response, Health, and Behavior

E Hutchinson, A Avery, S VandeWoude

Though environmental enrichment for laboratory animals is aimed at improving welfare, evidence suggests some enrichment practices may actually be harmful. Our laboratory previously examined mice raised in an un-enriched environment at a commercial vendor, but given multiple devices upon arrival at our facilities. Surprisingly, these mice experienced significant thymic atrophy and greater variation in parameters than their un-enriched counterparts, suggesting that enrichment resulted in a more stressful environment for mice reared in un-enriched housing conditions. This study aimed to verify these results and explorethe ffects of super-enrichment on a variety of parameters. Like our previous study, there were significant behavioral, physical, or immunologic differences between enrichment groups, suggesting that home cage environment from birth to weaning has long term effects on many parameters, and appears to enhance endogenous corticosterone production. Paradoxically, the presence of enrichment was correlated with fewer tail wounds signaling aggressive behavior. Future studies will eleaborate these findings in an attempt to define enrichment strategies most beneficial to mice.

Transplacental infection of bovine fetuses with non-cytopathic bovine viral diarrhea virus type II (BVDV-II): viral spread and brain lesions.

CE Reisenhauer, AE Tolney, N Smirnova,T Hansen, DL Montgomery, H Van Campen, H Bielefeldt-Ohmann.

Purpose: Despite the importance of fetal viral infections in both humans and animals, many questions regarding mechanisms of transplacental transmission, viral spread within the fetus, and consequences for target cells and the fetus as a whole remain unanswered. Infection with BVDV represents a reproducible natural model in which to address such questions. Materials & Methods: 18 BVDV-seronegative heifers were bred by AI. Six heifers were challenged intranasally with BVDV-II on day 75 of pregnancy, 6 at day 175 and 6 remained uninfected controls. Fetuses were retrieved at day 190 of pregnancy. Tissues were procured for histopathology, virus detection by qRT-PCR and by immunohistochemistry (IHC). IHC was also employed to assess the mechanisms of tissue damage, including apoptosis and cytokine cascades. Results: All 6 fetuses infected on day 75 of gestation were persistently infected (PI) with BVDV, and viral antigen could be detected in most organs examined. The primary target organs were brain, liver and spleen. In the brain viral antigen was detected in neurons, oligodendrocyte precursors and infiltrating macrophages. Histological changes included meningoencephalitis with leukomalacia, increase EMH in the liver and precocious development of lymphoid tissues. Fetuses infected on day 175 of gestation were negative for viral antigens and histologically unremarkable, as were all 6 control fetuses. By IHC the expression of HIF-1 was normal in the infected fetuses, but evidence of apoptosis was obtained by IHC for activated caspase-3. Conclusions: Infection of the bovine fetus late in the first trimester results in a persistent infection and will over time lead to neuropathology. Hypoxia does not appear to be central to the pathogenesis of the neural lesions, but viral induced apoptosis may play a role. Future studies will focus on the role of the oligodendrocytes and the fetal inflammatory response syndrome in the pathogenesis of the neuropathology.

Effect of iron overload on the pathogenicity of M. tuberculosis in the guinea pig.

LC Taraba, EA Smith, CA Shanley, CL Bedwell, IM Orme RJ Basaraba

The growth and virulence of M. tuberculosis depends on its ability to scavenge host iron, an essential and limited nutrient in vivo. Iron overload in humans has been associated with increased susceptibility to M. tuberculosis infection and excess iron has been shown to partially diminish the protective effect of M. bovis BCG (BCG) vaccination. The purpose of this study was to determine what effect iron overload had on the susceptibility of vaccinated and unvaccinated guinea pigs to M. tuberculosis. BCG-vaccinated and sham-vaccinated guinea pigs were pre-treated with iron dextran or dextran alone prior to aerosol challenge with M. tuberculosis. At 30 days post-infection animals were euthanized and tissues were collected to determine numbers of viable bacilli, iron concentration and lesion burden in lung, lymph node, liver and spleen. BCG vaccination significantly reduced pulmonary and extra-pulmonary lesion burden as well as numbers of culturable bacilli from all tissues examined. Iron loading had no significant effect on expression of disease in either BCG vaccinated or non-vaccinated animals. Despite the marked increase in intra-cellular ferric iron in liver and spleen of iron loaded animals, iron accumulation in lymph node and lung were moderate. In contrast to previous studies in mice and clinical observations in humans, iron loading had no significant effect on the pathogenicity of experimental M. tuberculosis infection in the guinea pig or on the ability of BCG vaccination to confer protection.

Recombinant Bovine Trypsin made in Maize Inactivates Bovine Herpes Virus-1 Adsorbed to the Bovine Zona Pellucida

ML Turk, GE Seidel, Jr., PW Gordy, RA Bowen

Purpose: Often it is desirable to use media for embryos that are devoid of animal products. However, the trypsin used for treatment of embryos for export is an animal product. Therefore, we studied the efficacy of recombinant trypsin produced from the bovine gene in maize (Trypzean™, Sigma T3568, St Louis, MO) for inactivating bovine herpes virus-1 (BHV-1). Materials/Methods: Active virus was titrated on MDBK cells using a standard plaque assay. Ova from superovulated cows were incubated for 45 min with BHV-1-infected cells for virus adsorbtion to the zona pellucida. Both unfertilized ova (N=40) and embryos (N=22) were aliquoted to 4 treatments: 1) control, no virus; 2) exposed to virus and washed 10X; 3) treatment 2 plus trypsin treatment as outlined in the IETS Manual; and 4) treatment 2 plus 525 U Trypzean/mL and 1 mM EDTA substituted for the 4th and 5th washes of 1 min each; the 6th of 10 washes was with soybean trypsin inhibitor at 80 mcg/mL (Sigma 93620). Ova were stored at -80°C until sonication and inoculation on to MDBK cells in duplicate undiluted and 0.1X dilution. The virus was allowed to adsorb for 45 min, and then overlaid with an MEM/agarose mixture followed by a similar mixture 2 days later; plaques were counted the next day. Results: Unfertilized ova and embryos led to similar results, which are pooled. No virus was detected in the 9 control ova not exposed to virus. As has been shown by others, BHV-1 remained adhered to zonae even after 10 washes (undiluted and diluted: mean of 36.5 and 3.8 plaque-forming units (pfu)/ovum). In contrast, treatment with Trypzean or the IETS trypsin protocols reduced this to near zero (undiluted and diluted trypsin treatment: 0.4 and 0.0 pfu/ovum; Trypzean treatment: 0.3 and 0.0 pfu/ovum). Conclusion: We thus confirm that trypsin treatment is effective in inactivating BHV-1 adhered to bovine zonae pellucidae, whether trypsin is derived from animals or genetically engineered plants.

Lectin dependent phagocytosis of virulent, type A F. tularensis.

SL Warner and CM Bosio

Francisella tularensis is an obligate intracellular bacterium that causes an acute, fatal pneumonia. F. tularensis possesses both a capsule and LPS rich in carbohydrates (CHO). Thus, CHO on the surface of F. tularensis may contribute to the uptake, and sub-sequentially virulence, of this bacterium. We demonstrate here that phagocytosis of a virulent, type A strain of F. tularensis (Schu4) by DC was dependent on lectin interactions. Phagocytosis of Schu4 by DC was independent of serum opsonins, but required divalent cations. Phagocytosis could be inhibited by zymosan, mannose, and laminarin, but not mannan. Interference with CHO lectins (Dectin-1, mannose receptor, or DC-SIGN) did not inhibit phagocytosis of Schu4. In contrast, blockage of CR4 (CD11c/CD18) significantly inhibited phagocytosis of Schu4. Collectively this data suggests an important role for CHO in the non-opsonic phagocytosis of virulent Francisella and that this process involves a direct interaction of the bacterium with the CR4 complex.

PVM: CLINICAL SCIENCE

Analysis of Dysplastic Cell Characteristics Found in Bone Marrow of Hematologically Normal Dogs

NR Abercrombie, AA Barker, JM Price

The purpose of this research was to conduct a retrospective study examining a series of bone marrow samples from hematologically normal dogs to establish a normal percentage of dysplastic myeloid, erythroid and megakaryocytic cells. This is essential to more accurately identify bone marrow samples from hematologically abnormal dogs. A 200 differential cell count was carried out on 20 randomly chosen archival bone marrow aspiration cytology samples from hematologically normal dogs. Normal erythroid and myeloid precursors were counted and any dysplastic cells in any of the cell lines were totaled. Erythroid dysplasia was defined as cells with nuclear or cytoplasmic abnormalities, nuclear mitotic figures, or asynchronous maturation. Myeloid dysplasia was defined as small myeloblasts or promyelocytes, giant metamyelocytes or band cells, or cells with asynchronous maturation, nuclear mitotic figures, or ring-shaped nuclei. In addition, 10 megarkaryocytes from each case were examined for hypolobulation. Each case was evaluated three times, each time by a different student and data were pooled and averaged for the development of reference intervals. 18 of 20 bone marrow cases had nuclear mitotic figures associated with the erythroid cell line, ranging from 0.165% to 2.75% of the total nucleated cell count. Other cell characteristics observed included giant metamyelocytes and band cells, ring-shaped nuclei and nuclear mitotic figures of the myeloid cell line, nuclear abnormalities and asynchronous maturation of erythroid cell line, and hypolobulation of megakaryocytes. These findings suggest some cell characteristics seen in dysmyelopoiesis may also be present in normal bone marrow. Therefore, we recommend further research that compares the amount of cellular abnormalities present in normal bone marrow cases to that of hematologically abnormal dogs to gain a more accurate understanding of their significance.

Early Postpartum Biochemical Parameters Related to Dairy Cow Removal

SM Hiibel, CS McConnel, JA Severidt, AE Hill, FB Garry.

Purpose: A large proportion of dairy cow deaths have been shown to be concentrated within the early postpartum period. The causes of death are related to the reasons for removal of cows for slaughter and most of these are representative of health issues. This study was designed to assess the value of standard biochemistry analysis for describing health problems associated with dairy cow removals (death and culling) during the early postpartum period. Materials/methods: This project was carried out on two intensive Colorado dairies. Serum was collected from cows at 3 to 5 days postpartum. Serum biochemistry panels for cows that were removed from the dairy within 30 days of parturition were compared with herd cohorts surviving through 100 days in milk, matched by calving date and lactation. For each biochemical parameter descriptive statistics were evaluated, and mean values in cases were compared to those in control animals using t-tests. Results: Biochemistry panels were run for 47 cows that were removed and 60 matched controls. Of the 19 parameters analyzed, only calcium (Ca), albumin, total bilirubin (TB), creatinine kinase (CK), and aspartate aminotransferase (AST) had significantly different means between cases and controls ($P < 0.05$). Ca and albumin were below normal in 36% and 17% of cases, and 25% and 7% of controls, respectively. TB, CK, and AST were above the standard range in 79%, 66%, and 53% of cases, and 40%, 22%, and 8% of controls, respectively. Conclusions: These findings suggest that electrolyte, musculoskeletal, gastrointestinal, and hepatic derangements are potentially key features of many cows that suffer premature removal during the early postpartum period. Appropriate sick cow therapy may be guided through adjunctive biochemical analysis. Biochemical analysis may also provide useful information highlighting areas of transition cow and calving management that require modification in an effort to improve postpartum health.

Biopsy-based 1-H and 31-P NMR shows regional metabolic heterogeneity within canine lymphoma

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Introduction: Metabolomics is the study of metabolite profiles of tissues obtained via nuclear magnetic resonance (NMR) and has potential to further understanding of cancer pathophysiology. Increased phosphocholine and phosphomonoester/phosphodiester (PME/PDE) are associated with malignant behavior. Increased lactate is associated with aerobic glycolysis, hypoxia and necrosis. Purpose: To determine if lymphoma (LSA) has a characteristic metabolic profile and to evaluate metabolic heterogeneity of samples relative to their location. Materials and Methods: Patients: Any dog with LSA, either sex, any breed and age. Tumors: Lymph node samples were taken via Tru-cut biopsy. For each tumor central and peripheral samples were taken and snap-frozen in liquid nitrogen. Extraction: All samples were processed for NMR by dual PCA/lipid extraction. Both water-soluble and lipid-soluble phases were extracted from each tissue sample. Proton and phosphorous quantitative NMR were performed. The PME/PDE ratio (1.46 µmol/g) and lactate values (3.52 µmol/g) in the LSA samples were similar to historical values for PME/PDE (1.53 µmol/g) and lactate (2.41 µmol/g) from a larger group of malignancies (n=11). In the LSA samples, the concentrations of lactate were 4.74 centrally and 1.9 peripherally and the PME/PDE ratios were 0.56 centrally and 2.4 peripherally. Results: In the LSA samples, the lactate concentrations were more than double in tumor centers and the PME/PDE ratios were significantly higher (4-fold) in tumor peripheries ($p<0.05$). Conclusions: This study demonstrated that LSA tumor samples had similar metabolic profiles relative to other malignancies, and also showed the value of biopsy-based NMR for mapping geographic metabolite differences. These data indicate a larger study is warranted to evaluate PME/PDE ratios and lactate as potential markers of malignancy, but there is a consistent geographic heterogeneity which emphasizes the importance of tracking sample location.

Development of a real-time PCR assay for the detection of pathogenic leptospires in canine urine

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Purpose: Leprospirosis is a zoonotic bacterial disease of worldwide importance. The aim of this study was to develop a real-time polymerase chain reaction (PCR) assay for detection of pathogenic leptospires in the urine of dogs. The development of this test for canine leptospirosis will enhance our ability to diagnose this disease and will allow comparison of the effects of different treatment protocols on shedding of leptospires in the urine of infected dogs. Materials & methods: The PCR primers and probe were generated using Beacon Designer Probe and Primer Designs Software. Primers were selected to amplify an 87 base pair product from a region of the Leptospira spp. 16S ribosomal DNA sequence. The fluorogenic probe included the reporter dye 6-carboxy-fluorescein at the 5' end, and the quencher dye 6-carboxy-tetramethyl-rhodamine at the 3' end. DNA was extracted from reference cultures of the pathogenic leptospiral serovars canicola, icterohaemorrhagiae, grippotyphosa, pomona, bratislava, and hardjo. Urine samples from a normal dog were spiked with aliquots of reference cultures of the serovars canicola, grippotyphosa, and pomona. The real-time PCR reactions were run on DNA isolated from the reference cultures and from the urine samples. All reactions were run on a BioRad iCylcer iQ. Results: The assay was positive for all 6 serovars, and product was detected over ranges of up to 6 ten-fold dilutions of the initial cultures. Product was detected from urine spiked with each of the three serovars used, over a range of 4-fold dilutions of the organisms in urine. The assay did not produce a detectable product from DNA isolated from Staphylococcus spp., Streptococcus spp., Pseudomonas spp., or E. coli spp. Conclusion: The results of present study indicate that this real-time PCR assay detects at least 6 leptospiral serovars that are pathogenic in dogs, and the assay is able to detect organisms in canine urine samples.

Effects of GnRH Immunization on Reproduction and Behavior in Female Rocky Mountain Elk

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Purpose: Overabundant wild ungulates cause problems when available habitats are insufficient, especially in ecosystems lacking natural predators. Managing this problem poses a challenge in protected areas, like National Parks, where traditional methods of wildlife management, such as hunting and culling, are not always feasible. While lethal removal may sometimes be necessary, there is also a need for effective, non-lethal methods of population control. Immunocontraception, using a novel anti-gonadotropin releasing hormone (GnRH) vaccine (GonaCon™), may provide a non-lethal alternative to traditional management practices in female Rocky Mountain elk (Cervus elaphus nelsoni). Materials/Methods: 10 captive female elk were treated with the GnRH vaccine at mid-gestation and 7 were treated with a sham vaccine to serve as controls. Measurements were taken throughout the remainder of gestation to determine serum progesterone concentrations, anti-GnRH antibody titers, injection site reactions, and calving rates. Blood samples from calves were also evaluated for anti-GnRH antibodies. Reproductive behavior was measured during the breeding season, and pregnancy rates were evaluated. In subsequent breeding seasons, the reversibility of the treatment will be evaluated. Results: Progesterone levels remained constant and were not different ($P<0.05$) between the treatment and control groups. Anti-GnRH antibody titers were greater than 1:1000 in 9 of 10 treated elk and were non-detectable in controls ($P<0.01$). Pregnancy did not occur in 9 of 10 treated animals, but all control animals became pregnant ($P<0.001$). Also, the anti-GnRH antibodies were passively transferred to calves through colostrum. Conclusions: Treatment with GonaCon™ vaccine during mid-gestation did not alter serum progesterone levels nor influence pregnancy. Additional studies are needed to determine the population level effects of the vaccine.

Neutrophil function in septic dogs

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Sepsis continues to be a major cause of morbidity and mortality in humans, foals, and canines. In septic human patients leukocytes appear to enter a hypoinflammatory state. Functional impairment of the innate immune response during sepsis has not yet been studied in dogs. The purpose of this study was to test the hypothesis that impaired neutrophil (PMN) function develops in response to severe bacterial infection in dogs. Thirteen dogs with clinical parameters consistent with sepsis were compared to 12 healthy control dogs. Flow cytometry combined with fluorescent markers allowed for quantification of PMN function; phagocytosis of E. coli, phagolysosomal oxidative burst, and intracellular reduced glutathione (GSH) concentration. Neutrophil phagocytosis of E. coli is significantly increased in septic compared to control dogs ($p<0.05$), and this is true whether a standardized reagent or autologous serum is used to opsonize the E. coli. The PMN phagocytic response was significantly greater ($p<0.05$) in both groups for E. coli opsonized with the standardized reagent, consistent with the presence of multiple serum factors that impact PMN function. The number of PMNs responding to E. coli phagocytosis with a maximal phagolysosomal oxidative burst is significantly less in septic compared to control dogs ($p<0.05$). There was no significant difference in PMN intracellular GSH levels. Certain components of the PMN response to bacterial infection appear to be enhanced in dogs with sepsis, while other parts of the response are subdued. Identifying significant changes in cytokines that control this part of the innate immune response may elucidate potential targets for pharmacological intervention in these critically-ill patients.

The lack of a stress leukogram as an indication of Addison's disease in the dog, a retrospective case study

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Purpose: To determine if the absence of a stress leukogram is a strong enough determinant of hypoadrenocorticism to suitably rule out other differentials in the diagnosis of a canine patient. Materials/Methods: Records for 100 dogs with diagnosed Addison's disease and 100 controls were pulled from Colorado State University's Veterinary Teaching Hospital, and the leukogram was analyzed. The lymphocyte counts were sorted to find a minimum and maximum value. A mean and standard deviation was taken and then analyzed with a z-test with a 95% confidence level. Proportions of patients with a lymphopenia, normal lymphocyte count, and a lymphocytosis were analyzed for both groups using a z-test with a 95% confidence level. Finally, a z-test with a 95% confidence level was used to analyze total proportion of animals in each category with a stress lymphopenia verses those with a normal or increased lymphocyte count. Results: Of the Addisonian patients; 74% had a lymphocyte count within normal limits and 22% had a lymphopenia. 58% of the control group had a normal lymphocyte count and 40% had a lymphopenia. A confidence interval of (.054, .306) was found comparing the proportions of stress lymphopenias between groups, and an interval of (.031, .289) was found comparing the proportions of lymphocytosis between the groups. Conclusions: Although the difference in number of patients lacking a stress lymphopenia between groups was smaller than expected, it does show that a normal lymphocyte count is a good predictor of an Addisonian patient. Of even more significance, this study showed that there is a statistical difference in actual lymphocyte counts between Addisonian dogs and other ill dogs. Therefore, an increase in the concentration of lymphocytes from a high normal value to a lymphocytosis is just as important an indicator for hypoadrenocorticism as the lack of a stress lymphopenia.

Nosocomial Syndrome Surveillance

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Purpose: To estimate the rate of occurrence of common nosocomial events among high-risk patients hospitalized at Colorado State University's veterinary teaching hospital. Materials/methods: This study included weaned canine and feline patients that spent some part of their hospitalization in the critical care unit and weaned equine inpatients that were admitted with clinical signs that were known or suspected to be related to the gastrointestinal system. At the time of each patient's discharge, a survey form was filled out by the primary clinician. The form included basic patient information as well as information about procedures and treatments the patient received. In addition to this, the clinician was asked to give their best clinical impression about whether one or more specifically defined nosocomial syndromes were recognized at any point during hospitalization.Results: 583 patients (502 small animal, 81 equine) were enrolled in the study over a 12-week period. 70 of those patients had a total of 86 nosocomial events reported (63 small animal, 7 equine). The nosocomial events were categorized as: GI disorders (24), IV catheter inflammation (22), surgical site inflammation (17), urinary tract infection/inflammation (7), septicemia (7), fever of unknown origin (6), and respiratory tract disorders (3). Conclusions: Results suggest that approximately 12% of hospitalized high-risk patients have recognizable nosocomial events. Further work is needed to identify the preventable fraction of these events and characterize the consequences and costs associated with them.

The association between causes of persistent hypercalcemia with the subsequent development of azotemia in dogs

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Purpose: Hypercalcemia is caused by numerous disorders in dogs. The most commonly reported cause is neoplasia, particularly lymphoma. Other less common causes include hypoadrenocorticism, chronic renal failure, primary hyperparathyroidism, hypervitaminosis D and chronic inflammatory disorders. Hypercalcemia can be a cause, as well as a result, of renal failure. The purpose of this study was to test the hypothesis that renal insufficiency, defined as azotemia, in persistently hypercalcemic dogs, is more frequent in dogs with humoral hypercalcemia of malignancy than other causes. Materials/Methods: A search of medical records at the Veterinary Medical Center from 2000-2006 revealed 104 dogs that were presented with persistent hypercalcemia without being azotemic. In each of these cases, signalment, biochemical profile values and approximate duration of hypercalcemia was reviewed. The cause of the hypercalcemia was determined, when possible, and development of azotemia secondary to the hypercalcemia was noted, when applicable. Results The most common causes of persistent hypercalcemia were lymphoproliferative disorders, (28 [27%]), primary hyperparathyroidism (26 [25%]) and anal gland apocrine cell adenocarcinoma (9 [9%]). Ultimately, 26 (25%) of the persistently hypercalcemic dogs became azotemic. Only hypercalcemia resulting from lymphoproliferative disorders (lymphoma or multiple myeloma) was associated with a significantly increased incidence of azotemia ($P = 0.009$). There was a significant difference in the incidence of azotemia in dogs diagnosed with primary hyperparathyroidism versus lymphoproliferative disorders ($P = 0.022$). No significant difference in the duration of hypercalcemia was detected between the two groups. Conclusions: Results suggest that hypercalcemia resulting from lymphoproliferative disorders is more likely to result in subsequent development of renal insufficiency than hypercalcemia due to other causes.

Identification of candidate genes involved in human XY abnormal gonad development.

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Human abnormal gonadal development occurs in approximately 1 in 15,000 live births. Although mutations in a number of genes (e.g., SRY, SF1, WT1) have been identified, the majority of XY gonadal dysgenesis cases are unexplained. Genes known to be required for proper fetal gonadal development are expressed in precursor somatic support cells (SSCs). SSCs give rise to Sertoli cells and granulosa cells of the mammalian testis and ovary, respectively. To identify new candidate genes required for gonadal development and differentiation, the transcriptional profile of isolated precursor SSCs was determined. Briefly, transgenic mice expressing a GFP reporter gene uniquely in XX and XY (pre)SSCs were used to isolated XX and XY pre-SSCs from undifferentiated fetal gonads by flow cytometry. The expression profile of orthologue mouse genes up-regulated in XY versus XX pre-SSCs was compared to known chromosomal regions involved in abnormal human XY gonadal development (i.e., pericentric region chromosome 5, terminal deletions chromosome 10q, and chromosome 17q21-24 (Meckel syndrome)). Using this approach, we identified 24 orthologue mouse genes up-regulated in XY pre-SSCs corresponding to regions of human chromosome 5, 10, and 17 associated with abnormal human XY gonadal development. Data obtained in these experiments provide a valuable resource to identify potential candidate genes involved in abnormal human testis development. Future experiments will examine the function of these candidate genes in testis development and differentiation.

Technetium-99M-Sestamibi Scans to Predict Outcome in Canine Osteosarcoma

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Purpose: This study was designed to correlate technetium-99m-sestamibi (MIBI) uptake with outcome in canine osteosarcoma (OSA). Hypothesis: Differential uptake of MIBI would be predictive for P-glycoprotein-mediated multiple drug resistance (MDR) and outcome in canine OSA. Methods: Thirty-five dogs presenting with appendicular OSA were staged to local disease only with thoracic radiographs and technetium-99m-hydroxymethylene diphosphonate scintigraphy. Patients were then scanned with MIBI and underwent amputation followed by doxorubicin-based chemotherapy and followed to failure. Four regions of interest were identified on the front and rear (tumor) leg of 17 dogs to identify a background isotope count and optimum test location based on the lowest coefficient of variation. The mean isotope count of the tumor region was divided by the mean background count to obtain a ratio. A similar evaluation was performed on the contra-lateral limb, to obtain a normal ratio. The normal ratio was subtracted from the tumor ratio to obtain a ratio difference. Disease-free interval (DFI) and survival time were compared between dogs having ratio differences above (>=) or below (<) the median ratio difference using Kaplan Meier product limit method. P-glycoprotein was measured using real time polymerase chain reaction (RT-PCR). Results: Twenty-eight of 35 cases were available for evaluation at a median follow-up of 78 months. The mean DFI and survival for dogs with MIBI uptake above the median (n=13) was 184 and 239 days, compared to 242 and 412 days for dogs with MIBI uptake below the median (n=15), respectively. These differences were not statistically significant. P-glycoprotein was not measureable in any of the samples analyzed. Conclusions: These results may suggest that MIBI could be a predictor of outcome, however, PGP-mediated MDR may not affect response to chemotherapy and outcome, at least as measured by technetium-99m-sestamibi uptake.

Efficacy of a Non-Replicating Subunit Vaccine Against Pulmonary Challenge with Virulent Yersinia pestis

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Purpose: Yersinia pestis is the causative agent of bubonic and pneumonic plague. Currently there is no approved vaccine for plague in the U.S. and earlier vaccines have failed to provide reliable protection against pneumonic plague. Therefore, we evaluated the efficacy of a vaccine composed of liposome-DNA complex (LDC) adjuvant and Y. pestis F1 capsular antigen for protection against pneumonic plague. Materials/ Methods: C57Bl/6 mice were vaccinated intranasally (IN), subcutaneously (SC), or orally (PO) with F1 antigen in LDC. After vaccination, serum was analyzed for titers of F1 specific IgG and IgA. T cell responses to F1 antigen were also assessed in the spleens and lungs of vaccinated mice. After immunization, mice were challenged IN with Y. pestis and survival and control of bacterial growth were assessed. Results: PO and SC immunization elicited high titers of F1 specific IgG and IgA antibodies, whereas IN vaccination elicited weak F1 specific antibody responses. When T cell responses were assessed, only SC vaccinated mice developed F1 specific IFN gamma producing T cells. Challenge studies revealed that IN immunization provided poor protection, 36% survival, while PO and SC immunization elicited greater protection, 92%. Immunization was also associated with control of bacterial dissemination and bacteremia. Protection appeared to be mediated by either CD4+ and/or CD8+ T cells, depending on the route of immunization. Conclusions: Our data indicate that protective immunity against lethal pneumonic plague can be achieved by oral immunization using a non-replicating subunit vaccine. Administration of the F1 antigen using the liposome-DNA complex adjuvant elicited protective immunity against rapidly fatal Y. pestis challenge. Both humoral and cellular immune responses appeared to be necessary for full protection. The results of these studies also suggest that it is possible to deliver a safe and effective orally administered subunit Y. pestis vaccine.

Plasma biochemistry values in dogs anesthetized for cardiopulmonary bypass - influence on anesthetic mortality

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Changes in plasma biochemical values and their influence on anesthetic mortality have not been described for clinical patients during cardiopulmonary bypass (CPB). Blood gas and electrolyte values, glucose, lactate, PCV, TP, and ACT from records of 35 dogs undergoing CPB between 2000 and 2006 were retrospectively analyzed. The population included 27 males, 8 females, 4.7±4.7years (mean ± SD), 22.8±13.1 kg anesthetized for surgical correction of congenital or acquired disease. Data were collected at 10 time periods, including: (1) post anesthesia induction (baseline); (2) before surgical incision; (3-8) during surgery and bypass; (9) end surgery; and (10) transfer to critical care unit (CCU). Data were analyzed by analysis of covariance with repeated measures. Effects were considered significant when $p<0.05$. Post-hoc comparisons were made between least squares means using t-tests. Data are reported as mean ± SD. Changes from baseline were seen for most parameters. The magnitude of these changes was generally greatest during CPB. For example, baseline glucose (mg/dL) and lactate averaged 103±22 and 1.3±0.5, respectively, and increased to 153±93 and 3.1±1.5 during CPB. Baseline PCV (%) and TP (g/dL) averaged 39±6 and 5.4±0.7, respectively, and decreased to 23±7 and 4.1±1.2 during CPB. Baseline Ca2+ (mmol/L) averaged 1.3±0.2.0 and decreased to 0.96±0.39; K+ increased to 4.85±1.73 from 3.2 ±0.6. Baseline ACT was 159±132 and increased to 1109±520 prior to CPB. Four of the 35 dogs died during the procedure, but no correlation was found between changes in individual blood chemistry values and outcome. Biochemical alterations during anesthesia and CPB can be explained by hemodilution, administration of cardioplegic solutions, and stress. Abnormal values returned to baseline with appropriate therapeutic interventions, and were not associated with increased risk of intraoperative mortality.